Effect of Psycho-Pharmacological Modulation of the Autonomic Nervous System on Human Oesophageal Pain Hypersensitivity

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Dr Claude Andrew Botha

School of Medicine & Dentistry

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Abstract

Background: Altered autonomic nervous system (ANS) function has been proposed as a mechanism in the development of central sensitisation (CS) and visceral pain hypersensitivity (VPH). The contribution of the parasympathetic nervous system (PNS) and the factors that mediate differences in sensitisation to acid are unclear and their study will clarify risk factors for oesophageal pain hypersensitivity (OPH) in gastro-oesophageal reflux disease.

Aims: To investigate psychophysiological and pharmacological manipulation of PNS tone in the development of OPH, and to determine factors which predict the development of OPH to acid infusion in healthy volunteers in a validated model of acid induced OPH.

Methods: Pain thresholds to electrical stimulation in the proximal oesophagus were determined before and after a 30-minute distal oesophageal infusion of 0.15 mol/L hydrochloric acid in subjects. Sympathetic (SNS) and PNS parameters were measured at baseline and continuously thereafter. Subjects underwent psychological profiling for anxiety, depression, attachment vulnerability and personality type. Using this model, five studies were undertaken: Study 1 a pilot study to trail modulation suitability for further study used. In Study 2, subjects who demonstrated secondary hyperalgesia in the proximal non-acid-exposed oesophagus performed deep or sham breathing. Study 3 subjects, who did not sensitise to acid, underwent a validated stress test to induce OPH. With Study 4, deep breathing with IV saline (placebo) or atropine (PNS antagonist) was used to evaluate deep breathing's induced PNS tone in OPH reduction. Study 5, a genetic pilot study, exploring the role of the GCH-1 haplotype in VPH.

Results: ANS control's key role in CS was clarified. Deep breathing increased PNS tone and prevented acid-induced OPH in comparison to sham breathing and confirmed increased PNS tone's reversal of OPH. Psychological factors of anxiety, alexithymia and attachment status influence ANS modulation of CS. Individuals' predisposition to VPH due to psychogenetic profiles were clarified and their biopsychosocial role illustrated.

Conclusions and Inferences: A mechanistic explanation for the analgesic effect of deep breathing is provided with potential therapeutic implications in the treatment of VPH syndromes. Further clinical study is warranted to develop cost-effective treatments for chronic VPH syndromes.

About the Author

The author was educated at Newcastle High School, Kwazulu-Natal and The University of Pretoria - School of Medicine & Dentistry, South Africa, where he was awarded the degree Bachelor of Medicine and Surgery in 1995. He completed his basic postgraduate medical training in London and gained membership of the Royal College of Psychiatrists in 2003, and concluded his higher specialist training in Psychiatry in 2009. He has been working as Clinical Fellow in Liaison Psychiatry of Gastroenterology at St Bartholomew's and The Princess Grace Hospitals since 2003.

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Statement of Originality

I, Claude Andrew Botha, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

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Details of collaboration and publications:

Two publications are at present under review, and collaborations are as stated below.

Declaration of Collaboration

The following individuals contributed to data contained in this thesis as stated below:

Study 4 (Chapter 5):

Matias Nilsson, **Christina Brock** & **Asbjørn Mohr Drewes**

Mech-Sense, Aalborg Hospital, Department of Gastroenterology, Aalborg, Denmark

Study 5 (Chapter 6):

David Bulmer

Wingate Institute of Neurogastroenterology, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London UK

Charles Mein

Genomic Centre, Charterhouse Square, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London UK

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List of Abbreviations

ACC – Anterior cingulate cortex
ACTH – Adrenocorticotrophic hormone
ANS – Autonomic nervous system
AVP – Arginine vasopressin
BFI – Big five inventory
BMI – Body mass index
BH4 – Tetrahydrobiopterin
BP – Blood pressure
CFS – Chronic fatigue syndrome
CGRP – Calcitonin gene related peptide
CNS – Central nervous system
CR – Coefficient of reproducibility
CRH – Corticotrophic releasing hormone
CS – Central sensitisation
CSB – Cardiac sensitivity to the baroreflex
CVC – Cardiac vagal control
CVT – Cardiac vagal tone
DAHP – 2,4-Diamino-6-hydroxypyrimidine
DMNX – Dorsal motor nucleus
DNA – Deoxyribose nucleic acid
DNIC – Diffuse noxious inhibitory control
DRG – Dorsal root ganglion
ECG – Electrocardiogram
EDTA – Ethylene-diamine-tetra-acetic acid
ENS – Enteric nervous system
EMS – Emotional motor system
EO – Erosive oesophagitis
FCP – Functional chest pain
FD – Functional dyspepsia
FGID – Functional gastrointestinal disorders

FH – Functional heartburn
FM – Fibromyalgia
fMRI – Functional magnetic resonance imaging
FSD – Functional somatic disorders
GABA – Gamma-aminobutyric acid
GCH-1 – Guanosine triphosphate
GI – Gastrointestinal
GORD – Gastro-oesophageal reflux disease
GSR – Galvanic skin responses
GTP – Guanosine triphosphate cyclohydrolase-1
5-HIAA – 5-hydroxyindoleacetic acid
5-HT – Serotonin (5-hydroxytryptamine)
HADS – Hospital Anxiety and Depression Scale
HF – High frequency
HPA – Hypothalamic pituitary adrenal
HR – Heart rate
HRV – Heart rate variability
IASP – International Association for the Study of Pain
IBS – Irritable bowel syndrome
ICC – Intra-class correlational coefficient
IPANs – Intrinsic primary afferent neurons
LF – Low frequency
LOS – Lower oesophageal sphincter
LVS – Linear vagal scale
MAP – Mean arterial blood pressure
MP – Myenteric plexus
MBP – Mean blood pressure
MCC – Mid-cingulate cortex
MEG – Magneto-encephalography
NA – Nucleus ambiguous
NCCP – Non-cardiac chest pain
NERD – Non-erosive reflux disorder
NG – Nodose ganglia

NMDA – N-methyl D-aspartate
NO – Nitric oxide
NTS – Nucleus of the solitary tract
OPH – Oesophageal pain hypersensitivity
PAG – Periaqueductal grey
PAR – Protease activated receptors
PB – Parabrachial nucleus of the dorsolateral pons
PET – Positron emission tomography
PFC – Prefrontal cortex
PGE2 – Prostaglandin E2
PI-IBS – Post infectious irritable bowel syndrome
PNS – Parasympathetic nervous system
PS – Peripheral sensitisation
PTT – Pain tolerance threshold
RBC – Red blood cells
RVM – Rostral ventral medulla
SA – Sino-atrial node
SI – Primary somatosensory cortex
SII – Secondary somatosensory cortex
SBP – Systolic blood pressure
SCR – Skin conductance responses
SEM – Standard error of the mean
SMP – Submucosal plexus
SNS – Sympathetic nervous system
SIDS – Sudden infant death syndrome
SUNDS – Sudden unexpected death syndrome
TAS-20 – Toronto Alexithymia Scale
UOS – Upper oesophageal sphincter
VASQ – Vulnerable Attachment Style Questionnaire
VPH – Visceral pain hypersensitivity
WAI – Weinberger Adjustment Inventory

1 Introduction

1.1 "One body-mind": a brief history

From the dawn of recorded history, emotions and the body were closely knit. To the ancient western and eastern cultures the body and the soul were one. Their literature bears witness to the antiquity of concepts, that the body in general and viscera in particular are core components of normal emotional life. (1) To the physician in the time of Hippocrates the 'medical model of the day' made no distinction between emotional and physical wellbeing. (2) Physical and mental concepts were freely interchangeable and equally relevant at procuring a diagnosis and cure. Present exclamations in the common vernacular; "lump in my throat", "heart skipped a beat" and "sick to the pit-of-my-stomach", etc. still attest to this notion. The Descartes-ian dualism (3) with the separation of the roles of brain (body) and emotion (mind), is a recent accretion however that in its quest to find something that lies '*beyond* all *doubt*', fostered the mistaken supposition of focusing on the physical body and consigning the mind (psyche) to a mere neglected epiphenomenon. (2, 4) This then regrettably gave rise to an epoch of misguided estrangement.

Further, extreme and persistent emotions/passions, in association with 'bodily-states' presently referred to as "stressed", "burn-out" or being "run-down" making specific individuals because of personal constitutional differences more vulnerable, has always been the received wisdom and can be traced in early written records ranging back to more than 4 thousand years ago.[1] (5) Only recently with the inclusion of concepts like the brain/mind by cognitive-neuroscience as a plausible reality deserving of serious research consideration, have there

[1] Galen in the 2nd Century BC

been early indications of the reparation of the ancient breach by modern scientific and clinical practice. It is with this mind-set, under the rubric of neurogastroenterology, that this thesis has been envisioned and pursued.

1.2 Modern neuroscience: "A fresh unified perspective"

From the 90's, the start of the "Decade of the Brain" until now, the integration of objective findings of an array of sciences profoundly deepened our understanding of the role relationships play in our day-to-day subjective lives. By exploring a wide range of sciences, from anthropology to neuroscience, and seeking the convergence of findings that emerges from their integration, one can arrive at a consilient[2] view of the "unity of knowledge" (or "consilience, as E.O. Wilson has used the term, 1998 (6)). In other words, as in the old tale of the three blind men and the elephant, there is a "larger reality" that exists though any single perspective can only begin to describe one view of that reality.

Through this present merger of several significant perspectives it has now been made possible and necessary for the field of neurophysiology to re-examine with "better tools" and "fresh eyes" the problem presented with regard to the interactions between pain, emotion and autonomic regulation. The main contributing factors making this possible and now needed include:

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[2] In the Brittanica Dictionary, consilience is defined as "the concurrence of generalisations from separate classes of facts in logical inductions so that one set of inductive laws is found to be in accord with another set of distinct derivation."

1) Great clinical need: Of late there is a significant increase of functional somatic syndromes that has placed a huge strain on an already over-stretched national healthcare system. Together with the fact that the pharmaceutical and academic industries were unable, in spite of significant investment, to arrive at a satisfactory improvement in diagnosing or treating these conditions, there has never before been a greater need for more effective and cost-effective management strategies of these poorly understood, and under-treated conditions as now.

2) Deeper understanding: This is made plausible due to novel conceptual heuristic frameworks, for example: (a) 'Bud' Craig (7) who has given the scientific community a clearer neurobiological basis from which to consider interactions between affect and pain, with the development of the concept of "homeostatic processing networks", allowing for the inclusion of sensory or the 'felt' aspects of pain. (b) Stephen Porges's (8) development of the "polyvagal theory" that has allowed us for the first time to truly understand the key psychophysiological responses of the motor outputs of pain/emotion responses in the brainstem vagal complex, and (c) "Interpersonal neurobiology" by Daniel Siegel (9), that combines a range of disciplines, from the interpersonal (communication, attachment theory and social psychology) to the neurobiological (psychobiology and the domains of affective, cognitive, and developmental neuroscience).

3) Improved Modern Technology: Technical developments in autonomic neuroscience with beat-to-beat (10) and breath-to-breath (11) selective cardiac vagal measures and beat-to-beat non-invasive blood pressure measures, has allowed for the real time analysis of the autonomic nervous system (ANS). This enables the analysis of live, *in vivo*, time

locked observations of physiological correlates that was hitherto obscured to previous investigators.

1.3 Syndromes of medically unexplained symptoms

1.3.1 Unexplained Medically Syndromes are common

A medical clinic in the USA in 1989 reviewed 1000 of their internal patients' case notes. (12) Kroenke and Mangelsdorff when recording the incidence of 10 commonly reported symptoms over a 3-year period found that the proportion of these symptoms attributed to "organic disease" compared to "unexplained", was (contrary to expectation) mostly due to "unexplained" aetiology. (White areas in each bar, figure 1)

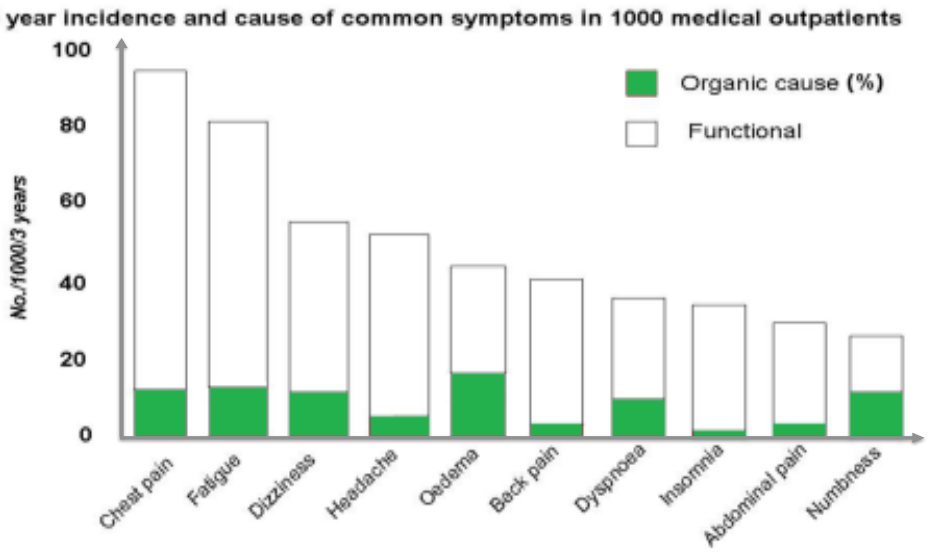


Figure 1 ***A case note review of 1000 patients, over a 3-year period to determine what proportion of symptoms, was attributed to "organic disease" or "unexplained" causes. The majority, across a several systems were due to functional aetiology.***
***(Adapted from Kroenke and Mangelsdorff, 1989)* (12)**

A similar finding was observed in a UK based study of secondary care cardiology, gastroenterology and neurology clinics, where only about

60% of patients attracted an "organic" diagnosis. (13) The remaining 40% were categorised as either "unexplained" or "functional". These unexplained conditions and resulting deficit in patient expectation are cited as a cause of significantly reduced patient satisfaction (14) and estranged doctor-patient relationships. (15) Chronic unexplained symptoms further contribute to impaired health related quality of life and is a health care burden leading to escalating costs. (16)

With regard to this, it is true that to a great extent patient culture determines the type of symptom and the severity that would warrant a medical consultation, with patho-physiology playing a secondary role. (17) But the reverse is also true, whereby physicians control the legitimisation of symptoms:

> "…*although biological and clinical factors have set boundaries for which symptoms might plausibly be linked in a disease concept, social influences have largely determined which symptom clusters have become diseases.*" (18)

In defining the term diseases, the present medical model would deem an underlying clinico-pathological change in tissue, e.g. by an infective agent or neoplasm, as the "medical-standard" for example. Symptoms that indicate an underlying disease state (as so defined) would therefore be acknowledged and distinguished from those that do not. (19)

Clinically, so doing, any condition "short of the mark", would consciously or unconsciously be deemed of less importance by the physician, and therefore stand a greater chance of being accompanied by an impudent clinical frustration, and attracting some negative stigma, with consequentially less validation and attention. In many instances, this would then evoke a counter response in the patients, where contrary to

being reassured by the medical consultation, they are left confused and frustrated, with an increased desire for validation, leading to increased consultation behaviour. The end result poses a picture where after several specialist referrals and countless costly special investigations, the "medical-standard" is still not met, and due to possible poly-pharmacy and poor inter-disciplinary communication, a lack of coherent case management leads to a further increase in health care costs (15) and a further decrease in the patient's quality of life. (14)

Academically however, varying combinations of symptoms, the so-called "functional syndromes" as opposed to merely symptoms, have become the main focus of modern research study. (19) Also various medical sub-specialties have claimed "ownership" of particular symptom clusters (syndromes), and in so doing facilitated the possibility of clinical 'tunnel-vision' and the potential masking of significant overlap between them, inhibiting exploration of common mechanisms. (19-21) The resulting on-going debates between the "lumpers and splitters" (22) has created an arguable false-dichotomy, whereas a proposed two-pronged combination of both levels of analysis may have greater practical applicability and clinical efficiency.

1.3.2 Functional Disorders in Gastroenterology

Specifically concerning gastroenterology, clusters of gut-focussed symptoms have similarly been designated as syndromes. (23) Progressive thinkers like for instance Drossman *et al.* (24) have given us the ability to consider multi-factorial aetiologies concerning these syndromes within a frame work like the "biopsychosocial model", which has successfully previously been applied to conditions e.g. peptic ulcer disease and ulcerative colitis. (25-31)

Unfortunately, this 'wide-berth' approach has given us an abundance of aetiological possibilities to consolidate, and hence initial intentions of unifying and integrating common mechanisms were waylaid. Further exacerbation of the aetiological diversity occurs when multiplied by subsequent organ-centric anatomical sub-divisions of functional syndromes, e.g. oesophagus: non-cardiac chest pain; stomach: functional dyspepsia and post-prandial distress syndrome etc., to name but a few. The underlining heterogeneity of these clinical divisions is reflected generally in their poor treatment response; with the only possible unifying indicator being the similarities regarding their high placebo response rates when compared to conditions like inflammatory disease. (32-34)

This highlights the psychological aspects, that when fully considered, potentially further 'muddies' the proverbial 'aetiological waters' regarding functional gastrointestinal disorders (FGID). For instance heritability-twin studies in irritable bowel syndrome (IBS), found that social learning contributed an equal or even greater influence than genetic heredity alone. (17) Similarly there is strong evidence increasingly suggesting daily environmental "life-stress" effecting IBS symptoms significantly. (35) The effective amelioration of some of these psychosocial contributors by means of psychotherapeutic interventions along with the previous findings has hence encouraged an increasing cognitive emphasis (and greater stigma) in their present FGID conceptualisation. (36, 37)

1.3.3 Functional Gastrointestinal Disorders – the need for further study

Concerning FGID, the most common presenting complaint is chronic episodic pain. These are conditions, such as Functional Dyspepsia, Irritable Bowel Syndrome (IBS) and Non-Cardiac Chest Pain (NCCP), being responsible for up to 40% of patients seen in secondary care gastrointestinal (GI) practice. These symptoms are the cause of significant morbidity. Health care costs related to them are approximately £21.5 billion[3] in the 7 largest western economies. (38, 39)

A third of patients with IBS give a previous history of gut inflammation or injury in the form of gastroenteritis or surgery. (40) The majority will recover with no further consequences however a proportion may develop chronic unexplained pain. Furthermore, patients are more likely to develop chronic symptoms if they have increased psychosocial stress at the time of injury/inflammation. (41) Similarly, 25-60% of patients with NCCP have evidence of gastro-oesophageal reflux disease, with the remainder being classified as having Functional Chest Pain of presumed oesophageal origin. (42) Although no acid exposure can be documented in these latter subjects, it is possible that previous chemical exposure has resulted in heightened pain sensitivity, and significantly contributes to persistent poor treatment outcomes.

In patients with FGID, visceral pain hypersensitivity (VPH) is thought to be an important mechanism in the development of chronic pain, (43) however the factors that predict the development of chronic pain or VPH in these patients after inflammation or injury to the GI tract are not well understood. For instance the precise mechanisms for inter-individual

[3] $34 billion: Study done for the costs in the year 2000.

differences in the tendency to develop VPH after gut inflammation or injury are often difficult to identify. In addition to the severity of the external stressor (e.g. bacterial virulence, degree of inflammation and magnitude of injury), hosts of factors such as psychological state and trait, genotype, early life experiences and physiological factors such as bio-mechanical properties of the gut are also likely to be important.

As the pathophysiology is multifactorial there is evidence to suggest that psychological processes have a role in these disorders (44) as 60% of patients with FGID have tended to have a history of psychosocial stress, anxiety or mood disorders. Studies have shown that patients with the highest levels of psychosocial disturbance tended to suffer from the most FGID syndromes. (45) This information indicates the importance and emphasises the need for more accurate psychological profiling, as bio-psychosocial elements are integral in influencing predisposition to these disorders.

1.3.4 "Functional Neural Disturbance" - Time for a new focus

"Functional neural disturbance" is not a new idea. As a concept, Charcot, Willis and Beard have postulated it already in the 20th century. An increasingly dualistic biomedical model in the intervening century discriminated between the physical and the psychological (24), so that the "purely psychological illness" was firmly imbedded in later literature by figures such as Babinski and Freud. (19) This gradually acquired a pejorative stigma in which the description of physical symptoms being "all in the mind" was unacceptable, offensive and behaviourally rejected by patients. (46) For the physician and researcher alike, this was also not satisfactory as:

> *"Traditional psychosomatic models had less predictive value and less therapeutic importance than what was hoped for. The main problem with these models was the lack of a pathophysiological explanation for why psychological problems could be related to somatic disease…"* (47)

According to "symptom researchers" Sharpe and Carson, what is now required in research and clinical practice is greater integration by means of a shift in focus (the so-called "*paradigm shift*" (19)), whereby "functional disturbance of the nervous system" becomes the main investigative focus of unexplained medical symptoms, as:

> *"The combination of cognitive psychology and neurophysiology offers a model… for the understanding of subjective complaints and illness."* (47)

The conception of functional neural disturbances allows a less stigmatised "all in the brain" approach that also facilitates more biologically based research. Similarly a

> "…*new intellectual framework for psychiatry" has emphasised that "there can be no changes in behaviour that are not reflected in the nervous system and no persistent changes in the nervous system that are not reflected in structural changes on some level of resolution… …all mental processes are biological, and therefore any alteration in those processes is necessarily organic."* (48, 49)

Although in terms of originality the notion of a functional neural disturbance is by no means novel, nonetheless there have been several conceptual and technological developments since the time of Charcot, which allow for a more sophisticated integrative approach. (50) In particular the discovery of genes, neurotransmitters and functional

imaging; a deepened understanding of the basic science of nociceptors, the autonomic, neuroendocrine and central nervous systems; and epidemiological evidence for modulation by psychiatric illness and the neurodevelopmental effects of abuse have all converged to make this a timely opportunity to revisit the notion of functional neural disturbance; especially pain hypersensitivity, as it is one of the most commonly cited reasons for patient presentation.

1.4 Visceral pain hypersensitivity

Related to the idea of a "functional neural disturbance" is "visceral pain hypersensitivity". This is based on a proposed mechanism underlying unexplained visceral pain by means of spinal, dorsal-horn mediated "central & peripheral nervous system sensitisation", which has recently become the most likely and preferred explanation for observed data. (51-54) It displaces the previous out-dated incumbent descriptions of visceral motoric disturbances such as "gastric spasm". (55)

1.4.1 Mechanisms of pain hypersensitivity

Research in somatic pain hypersensitivity has suggested that both peripheral and central mechanisms can increase nociceptive transmission following inflammation or injury to tissues. (Figure 2) Peripheral mechanisms include peripheral sensitisation (PS), which is an inflammatory mediator-induced reduction in the transduction threshold of nociceptor primary afferents. PS causes pain hypersensitivity at the site of injury or inflammation, also known as primary hyperalgesia. (56, 57) Pain hypersensitivity that occurs in the surrounding healthy tissues (secondary hyperalgesia) and is related to an increase in excitability of spinal dorsal horn neurones due to upregulation of N-Methyl-D-Aspartate

(NMDA) receptors, a phenomena termed central sensitisation (CS). Both PS and CS are the major mechanisms in the development of inflammatory and neuropathic pain.

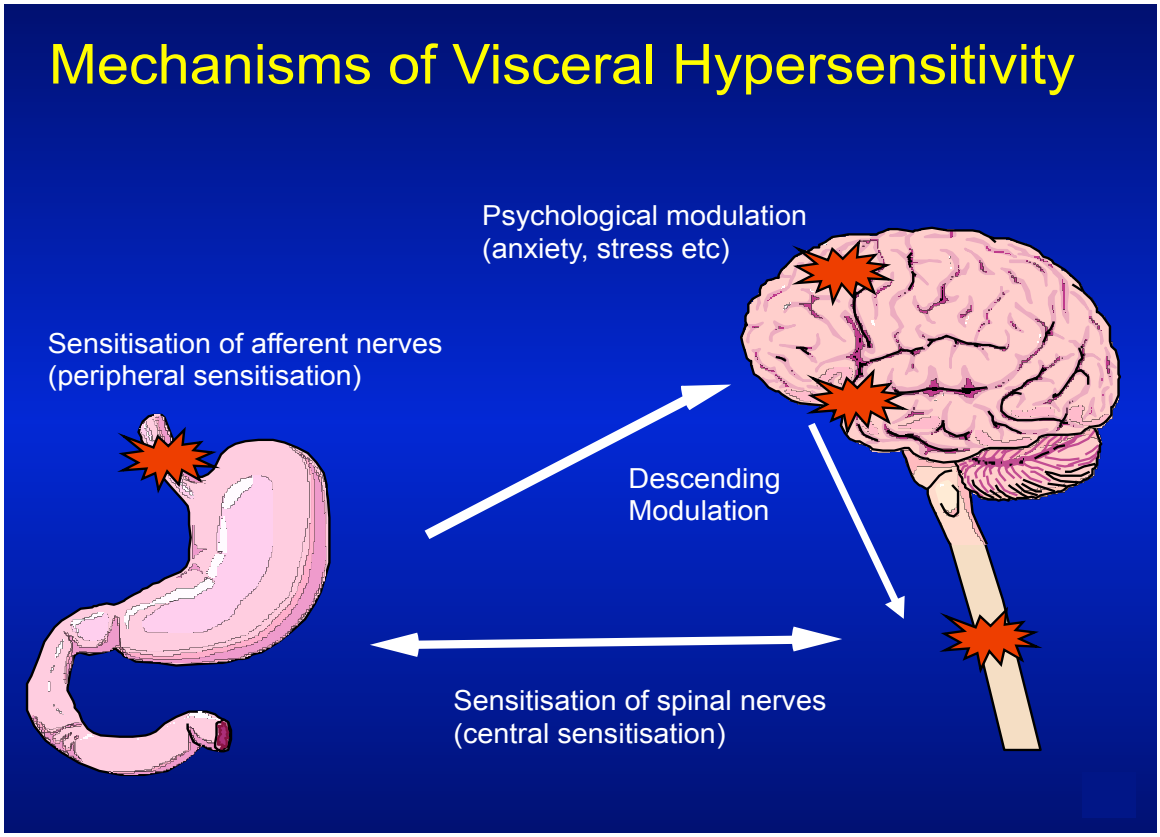


Figure 2 ***Schematic depiction of the mechanisms and interactions of peripheral and central sensitisation contributing to oesophageal hypersensitivity.***
***(Adapted from Aziz, 2000)* (58)**

1.4.2 Peripheral and central sensitisation in the GI tract

In the peripheral nociceptive nerve terminals, sequential activation of receptors leads to an increased membrane potential which increases peripheral axonal firing (Figure 3). This has an effect on the peripheral nociceptor sensitisation at a molecular level. This is brought about by means of several mechanisms, including a decreased transduction threshold, upregulation of ion channel expression and bidirectional neuroimmune interactions. Repetitive firing of action potentials from the

periphery also activates intracellular signalling cascades within the spinal dorsal horn neurons. This leads to amplified responses to both noxious – hyperalgesia and innocuous stimuli – allodynia. In figure 1.3 and 1.4, Knowles and Aziz (59) give an apt illustration and description of the mechanisms of PS and CS at the molecular level.

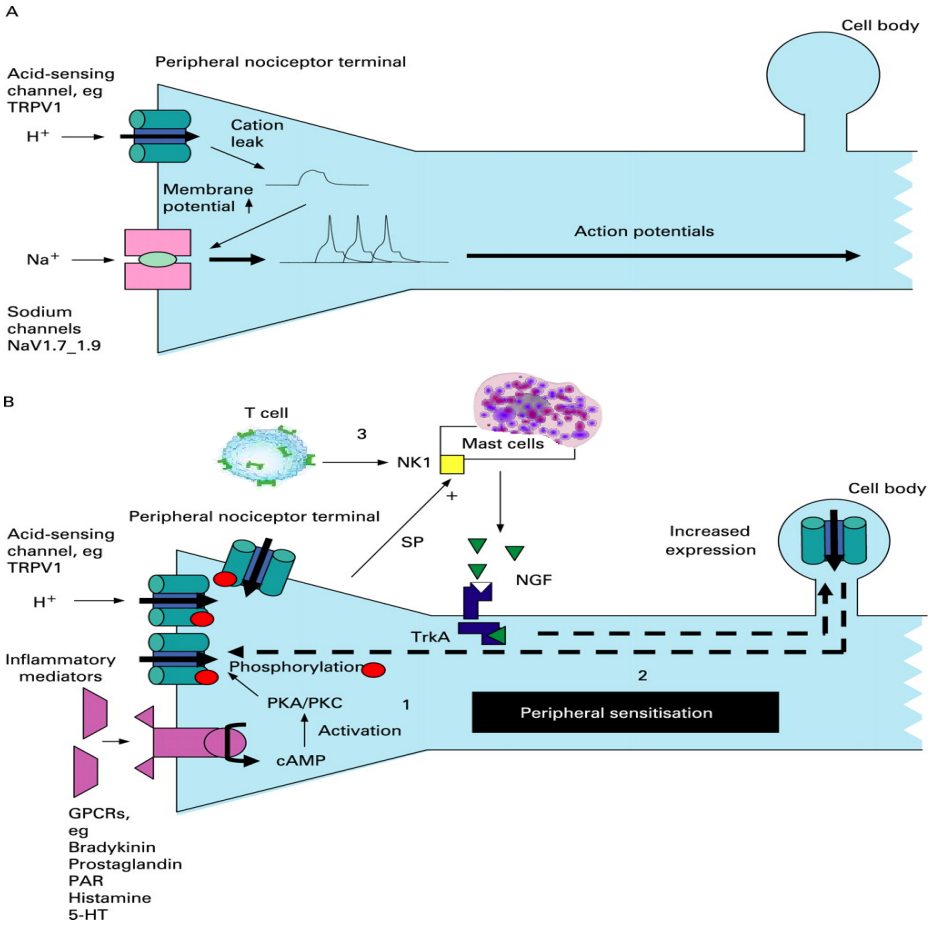


Figure 3 ***A: Schematic demonstration of action potential generation in nociceptors in response to acid stimulus. Cations are caused to leak from a variety of acid-sensitive ion channels—for example, the transient receptor potential channel 1 (TRPV1)— leading in turn to an increased (less negative) membrane potential. This causes sequential activation of nociceptive (selectively expressed by nociceptors) sodium channels NaV1.7–1.9 and axonal firing. B: Mechanisms of nociceptor sensitisation (including to acid): (1) decreased transduction threshold by phosphorylation of ion channels (mediated by intracellular activation of protein kinases in response to G-protein-coupled release of cAMP); (2) upregulation of ion channel expression—for example, TRPV1 in response to trophins—for example, to nerve growth factor (NGF) with retrograde transport from the cell body to nerve terminals; (3) bidirectional neuroimmune interactions, especially in respect of neuronal substance-P (SP) release acting on mast cells to release NGF. GPCR. G-protein-coupled receptor; 5-HT, 5-hydroxytryptamine; NK1, neurokinin 1; PAR, protease-acivated receptor; PKA, protein kinase A; PKC, protein kinase C; TrkA, tyrosine kinase receptor A.***

(Figure and text from Knowles and Aziz, 2008) (59)

Direct evidence for PS as a mechanism for VPH is mainly obtained from animal studies where inflammation involving primary nerves leads to a reduction in their transduction threshold. In humans the evidence is more indirect. For instance, patients with post-infectious - irritable bowel syndrome (PI-IBS) and non-erosive reflux disorder (NERD) demonstrate VPH and evidence of microscopic inflammation in the colonic and oesophageal mucosa respectively, despite absence of macroscopic changes. In the oesophagus at least these changes appear to more prominent when psychological stress is concomitantly present.

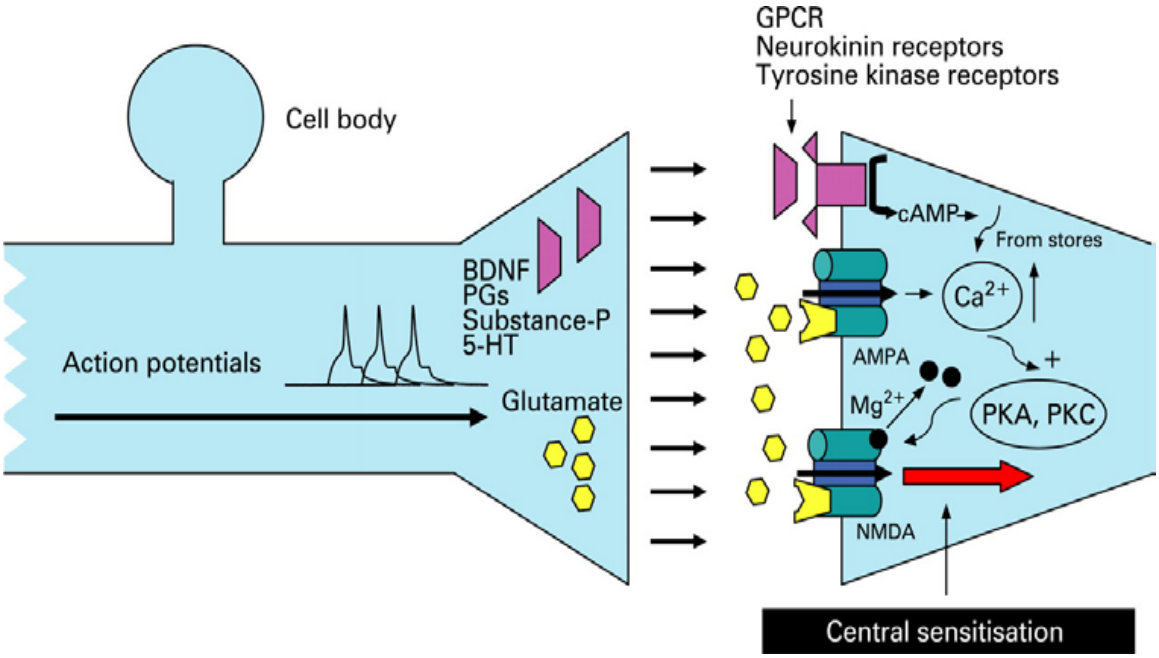


Figure 4 ***Molecular mechanisms of central sensitisation. Incoming action potentials lead to release of various neurotransmitters and neuromodulators that act via G-protein-coupled receptors (GPCRs) (prostaglandins (PGs), 5- hydroxytryptamine (5-HT)), neurokinin recptors (substance-P) and tyrosine kinase (brain-derived neurotrophic factor (BDNF)) as well as ligand-gated ion channels (glutamate). Subsequent intracellular messaging systems (predominantly via increased intracellular calcium and activation of protein kinases A and C lead to phosphorylation of N-methyl-Daspartate (NMDA) receptors with a reduction in voltage-dependent magnesium block. This potentiates its responsiveness to glutamate and leads to central sensitisation in the neuron concerned and those adjacent to it (secondary hyperalgesia). AMPA, a-amino-5-hydroxy-3-methyl-4-isoxazole proprionic acid; PKA, protein kinase A; PKC, protein kinase C.*** ***(Figure and text from Knowles and Aziz, 2008) (59)***

Evidence for a role of CS as a mechanism for the development and maintenance of visceral pain hypersensitivity comes from both animal and human studies. (60) Animal studies have demonstrated that following somatic inflammation a positive correlation exists between visceral pain thresholds and increased afferent discharge of dorsal horn neurones demonstrating viscerosomatic convergence. Spinal cFOS expression, used as a marker of dorsal horn activity, has also been shown to be increased following noxious colorectal distension and this is prevented by NMDA receptor antagonism. (59, 60)

It is clear from the above description that peripheral inflammation or injury can indeed cause PS and CS. However, it is not clear why chronic pain and hypersensitivity only develops in a relatively small proportion of patients exposed to such influences. It also seems that there is an interaction between the psychological state and the development of chronic pain hypersensitivity. How and why this interaction occurs is not clear. It is however possible that this interaction is mediated via the autonomic nervous system (ANS). (61)

1.4.3 Visceral Pain Hypersensitivity as biomarker

Although individual variability of visceral hypersensitivity of pain thresholds exists at a group level, however it has little clinical utility as a discriminatory biomarker. The sensitisation concept is not confined to IBS research, nor even to the field of pain, and as such could turn out to be but a subset of medically unexplained symptoms. The limiting narrow definition of the spinal dorsal horn mechanism of sensitisation could in time give way to a broader concept of generalised neural/psychological sensitisation, which may have more widespread relevance and applicability. (22, 47, 50, 62) An example of this is for

instance the recent emphasis of inhibitory and disinhibitory mechanisms of hierarchical neural control over sensitisation. (63) Recently individual variation in the modulation of activating and inhibitory neural systems have been proposed as the fundamental basis of inter-individual differences in sensitisation, and so may have greater promise for the development of discriminatory biomarkers. (64-66) A more detailed understanding of the biological underpinnings of emotion and its interactive control thus becomes necessary.

1.5 Homeostasis

Barnard & Cannon (67) proposed the concept of homeostasis or "*maintenance of the 'interieur milieu'",* which is a fundamental organising principle in understanding any physiological process. This is of particular importance when considering the complex physiological processes such as somatic and visceral perception giving rise to symptoms. This original idea has been developed to include regulatory concepts like "homeodynamic regulation", "allostasis" and presently "allodynamic regulation". They are dynamic systems without fixed points or fixed operating characteristics and with individual differences in expression. (68) They include the notion that:

> *"Physiological changes associated with behavioural states may reflect the active inhibition of set-point regulation – and not adoption of an altered regulatory level"*. (69)

However, homeostasis remains the instantly recognisable key-concept of fundamental physiological self-regulation.

Craig *et al.* (68, 69) describes homeostasis as comprising of 3 fundamental processes:

1) Detecting the inner needs of the organism (interoception),

2) Detecting external environmental sources of supply or threat to those inner needs (exteroception) and,
3) Moving towards or away from the external source appropriately.

For simpler organisms, chemical or chemico-humeral mechanisms are sufficient to form the basic response mechanism. The next phylogenetic stage utilises the immune system and finally develops a neural system that increasingly becomes more elaborate and sophisticated. (70)

As the 3rd step consists of reflex tropisms in the simplest organisms, unsophisticated response apparatus are sufficient for steps 1 and 2. As organisms differentiate in increasing complexity, greater amounts of appraisal occur in steps 1 and 2, and step 3 requires more executive control. Consequently the sensory, motor and motivational apparatus increases in sophistication. Finally, as individual survival becomes more contingent on survival within-and-of the group, the neuro-endocrine and immune systems become increasingly orientated towards social homeostatic requirements. (70) Inter personal psychology, in the making and maintaining of inter group relationships etc., then starts to play a role of growing importance in the homeostatic maintenance of the individual.

Comparative functional anatomy studies indicate that across the phylogenetic spectrum, all vertebrates share the same basic homeostatic mechanisms; only the complexity increases and reaches its peak in man. As these mechanisms increase in complexity, it becomes less obvious that the basic function of these systems is survival of the individual through homeostasis.

This is particularly the case when considering processes such as emotion, memory, social behaviour and even consciousness. These can seem distant from basic homeostatic physiological processes such as digestion (71) and yet they are based on and developed from these fundamental physiological processes and serve the function of homeostasis, as they are still intrinsically connected and dependent on them. To acknowledge this fundamental function and these simple mechanisms is not to depreciate emotion, attention, social behaviour and consciousness as in man they do not *solely* or *directly* serve *only* the process of homeostasis. As it became increasingly true that

> "…*to survive, mankind was not only dependent on 'survival of the fittest', but rather 'survival of the [best] nurtured'*". (9)

To recognise that they share similar physiological "circuitry" derived from homeostatic necessity, aids in explaining experimental data and facilitates further exploratory research as these seemingly transient processes have some knowable and measurable physiological substrate.

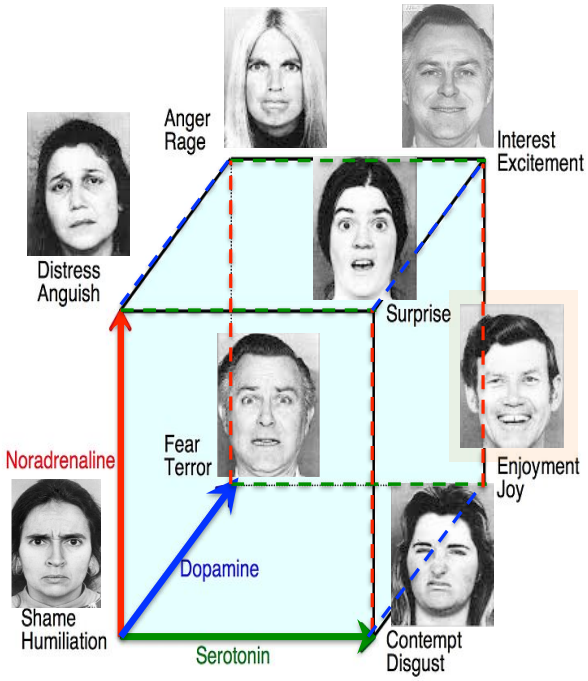
1.6. Emotion and Pain

1.6.1 Definitions and Dimensions of Emotion and Pain

In philosophy and cognitive-neuroscience, emotion is defined as:

> "…*a subjective, conscious experience that is characterised primarily by psychophysiological expressions, biological reactions, and mental states. Emotion is often associated and considered reciprocally influential with mood, temperament, personality, disposition, and motivation, as well as influenced by hormones and neurotransmitters such as dopamine, noradrenaline, serotonin, oxytocin, cortisol and GABA.*" (72)

Emotion is often the driving force behind motivation, positive or negative. (73) Definitions of emotion abound but most include a (i) felt/sensory (affect) component, which is significantly neurotransmitter mediated (e.g. Lövheim's cube, see figure 5) and (ii) motor/motivational/behavioural components. Emotions are usually arranged along the dimensions of hedonic valence (approach/avoid) and levels of physiological arousal. (74)



Basic emotion	Serotonin	Dopamine	Noradrenaline
Shame/humiliation	Low	Low	Low
Distress/anguish	Low	Low	High
Fear/terror	Low	High	Low
Anger/rage	Low	High	High
Contempt/disgust	High	Low	Low
Surprise	High	Low	High
Enjoyment/Joy	High	High	Low
Interest/excitement	High	High	High

Figure 5 Lövheim's cube of emotion; a three-dimensional model of emotion and monoamine neurotransmitters. Lövheim proposed a direct relation between specific combinations of the levels of the signal substances dopamine, noradrenaline and serotonin and eight basic emotions. A model was presented where the signal substances form the axes of a coordinate system, and the eight basic emotions according to Silvan Tomkins are placed in the eight corners. So joy/enjoyment is, according to the model, for example produced by the combination of high serotonin, high dopamine and low noradrenaline. (Adapted from Lövheim, 2012) (75)

The International Association for the Study of Pain (IASP) defines pain as

> *"...an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."*(76)

Pain is a complex multidimensional psychophysiological phenomenon comprising of sensory-discriminative, motivational-affective and cognitive-evaluative dimensions together with behavioural and physiological responses, and is conceptualised within the pain neuromatrix as proposed by Melzac *et al.* (77, 78) (Figure 6).

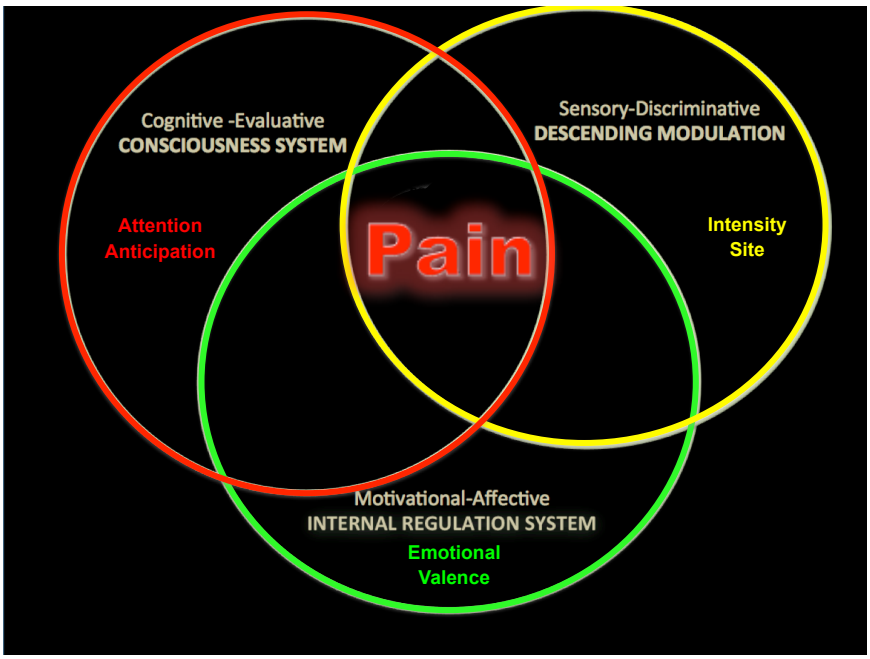


Figure 6 *The components of pain neuromatrix as proposed by Melzac et al. (Adapted from Melzack and Casey, 1968 & Melzack and Kat, 1999)* (77, 78)

1.6.2 Neural processing of Emotion and Pain

Damasio (3) has recently contributed substantial evidence for the key role played by "somatic markers" in neural processing, including that of emotion and pain. He indicated that the mind depends on "brain-body

interactions" for a wide spectrum of cognitive functions ranging from unconscious/automatic maintenance of homeostasis, experienced sensation and emotion, and even the regulating of higher-order reasoning and decision-making. In all these interactions, unconscious automaticity is the main *modus operandi* in their regulation. This is true for emotional processing (79-82), facial processing (83) as well as visceral afferent and efferent activity. (67, 84) Consciousness, being volitional, is almost the single exclusion.[4] (85) Figure 7 is derived from PET visceral pain studies showing that the majority of brain sites involved in emotion generation and processing are sub-cortical and generally associated with automatic processes.

Hence when applied to the pain neuromatrix, processing is localised as follows: Sensory- discriminative component of the pain experience relates to the localisation and intensity rating of the sensation (Figure 7: S1 & S2), whereas the affective-motivational aspects relating to its unpleasantness gives rise to emotional aspects such as fear and the formation of implicit- "felt"(visceral) memories (Figure 7: insula cortex, limbic & subcortical structures). Cognitive evaluative aspects facilitate the interpretation and contextualisation of pain and are thus involved in attention, anticipation and formation of the explicit- "narrative" memories of the experience. (Figure 7: Pre-frontal and anterior cingulate cortices) (86)

[4] Bargh *et al.* (1999) has estimated that only 5% of human behaviour is consciously determined, and coined the phrase "the unbearable automaticity of being!" as a result.

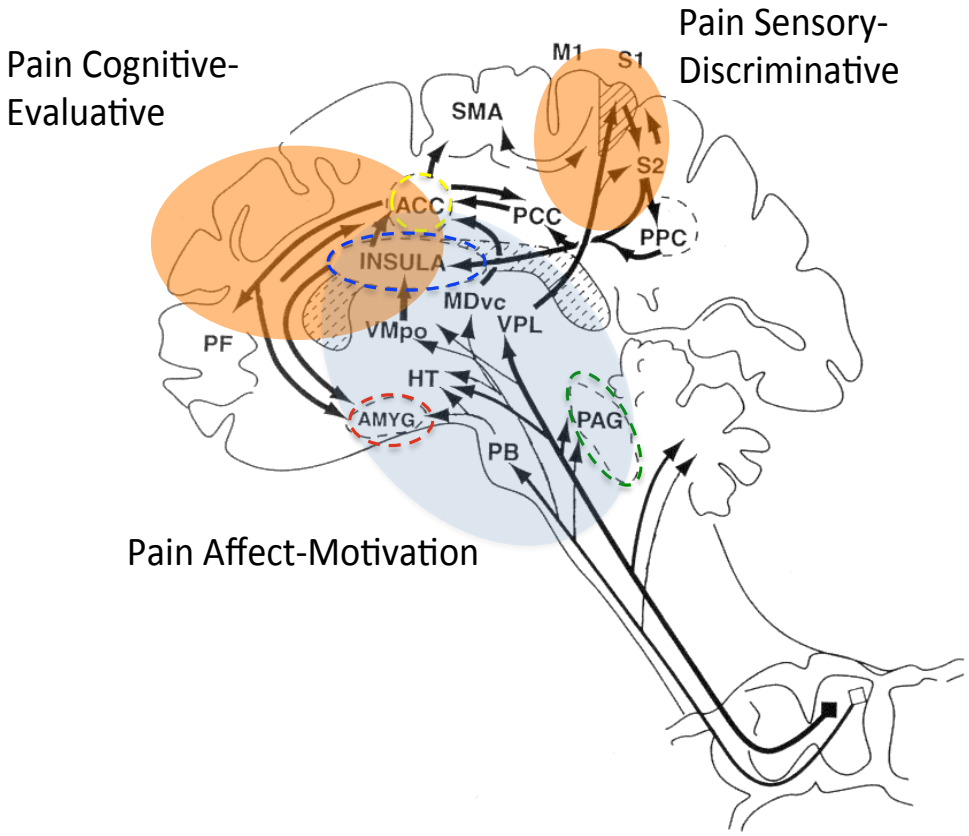


Figure 7 ***Shows the subcortical and cortical structures that have been shown to be activated in response to visceral pain. Abbreviations: PAG, periaqueductal gray; PB, parabrachial nucleus of the dorsolateral pons; VMpo, ventromedial part of the posterior thalamic nuclear complex; MDvc, ventrocaudal part of the medial thalamic dorsal nucleus; VPL, ventroposterior lateral thalamic nucleus; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; HT, hypothalamus; S1, S2, first and second somatosensory cortical areas, respectively; PPC, posterior parietal complex; SMA, supplementary motor area; AMYG, amygdala; PF, prefrontal cortex; M1, motor cortex. (Adapted from Price, 2000) (87)***

1.6.3 Psychophysiological "over-lap" of Emotion and Pain

Because pain overlaps and interacts with a diverse range of emotions, with a wide scope stretching from satiety to fear, (88) pain has recently been redefined as a "homeostatic emotion". "Homeostatic emotions" share psychological, anatomical and physiological features including cortical and sub-cortical substrates. Clinically it is observed that pain is one of the most common presenting complaints, irrespective of it being

explained or unexplained. (89) But equally chronic visceral pain conditions, such as FGID's, are frequently co-morbid with affective disorders such as anxiety and depression. (37, 90)

Structures especially implicated, as discussed above, are the so-called "interoceptive" insula cortex and the "homeostatic motor" anterior cingulate cortex. (7) A common effector for homeostatic emotions is the "emotional motor system" (EMS), which includes the central autonomic structures, peripheral autonomic network, and the neuro-endocrine-immune systems. (88) Thayer and Lane (91) in reviewing the literature for these systems, concluded that the Central Autonomic Network (92, 93); the Anterior Executive Region also called the "rostral limbic system" involved in "assessing the motivational content of internal and external stimuli and regulating context-dependent behaviours"(94); and the "emotion circuit"(95),

> *"…are one and the same functional network identified by different researchers from differing orientations. This network of CNS structures is associated with the processes of response organisation and selection and serves to modulate psychophysiological resources in attention and emotion".* (91)

Thus these frontal-subcortical structures, which were consistently activated during the PET visceral pain studies discussed in the previous section, consistently over-lap, or are the very structures regulating "executive, social and motivated behaviours". (96-98) As such these represent key central structures in homeostatic functional neural networks, which are likely to be of fundamental importance for psychophysiological processes and its behavioural assessment.

1.6. Psycho Motivational Behaviour of Emotion and Pain

Porges has provided compelling evidence for the central role of the ventral vagal nuclei within the right nucleus ambiguus in emotion and pain responses. He highlighted the special connection of the fifth and seventh cranial nerves in the homeostatic functional neural network. This has led some researchers to include facial expression as "visceral" in nature in distinction from other somatic responses. (99, 100)

Charles Darwin was first to observe that facial expression is unique to mammals and especially the primates and proposed that together with language plays a key role in both the expression and perception of emotional and hence homeostatic processes. (101, 102) In simpler organisms the homeostatic process is binary and behaviourally obvious: the organism either moves towards (approach) external sources (e.g. food or a potential mate) which can meet homeostatic need, or moves away from (avoid) external sources (e.g. a predator or pain) which represent threat. This binary axis underpins the dimensions of emotion: valence (aversive vs. appetitive) and arousal (increased vs. decreased). The overt behavioural responses of approach or avoidance are thus determined by the relative blends of valence and arousal. (74, 103)

The behavioural concomitants are less clear in humans, as social homeostatic requirements are much more complex, developing to the extent where they can override more basic physiological homeostatic functions (for example: courage, empathy, jealousy or spite). In this context where the responses are affective-motivational by nature and not behaviourally overt, the notions of approach-avoidance is less helpful, and should rather be considered in terms of engagement or disengagement. (99) Due to ambivalence, assessment of valence can

be further blurred, and also needs to be factored in when considering the physiological concomitance of human-environmental interactions.

In response to this, the assessment of individual emotional and personality differences of pain/threat defence response systems have been explicitly incorporated in the neurobiologically revised version of personality by Gray and McNaughton. (64-66, 104-106) For this thesis this is therefore a particularly important model in tying together relating "surface traits", with individual differences in underlying behavioural activation, inhibition and defence systems as well as the "emotional" valence dimensions of reward/safety and punishment/threat. The model also relates to conditioning, arousal and attention.

According to them, the behavioural inhibition system has a superior position in the hierarchy influencing decision-making and conflict detection. In reviewing defensive behaviours, they distinguish between a variety of possible activation states ranging between (i) classic flight-freeze behaviour (Figure 8 A) and (ii) a repertoire of "defensive approach" or "risk assessment" behaviours (Figure 8 B).

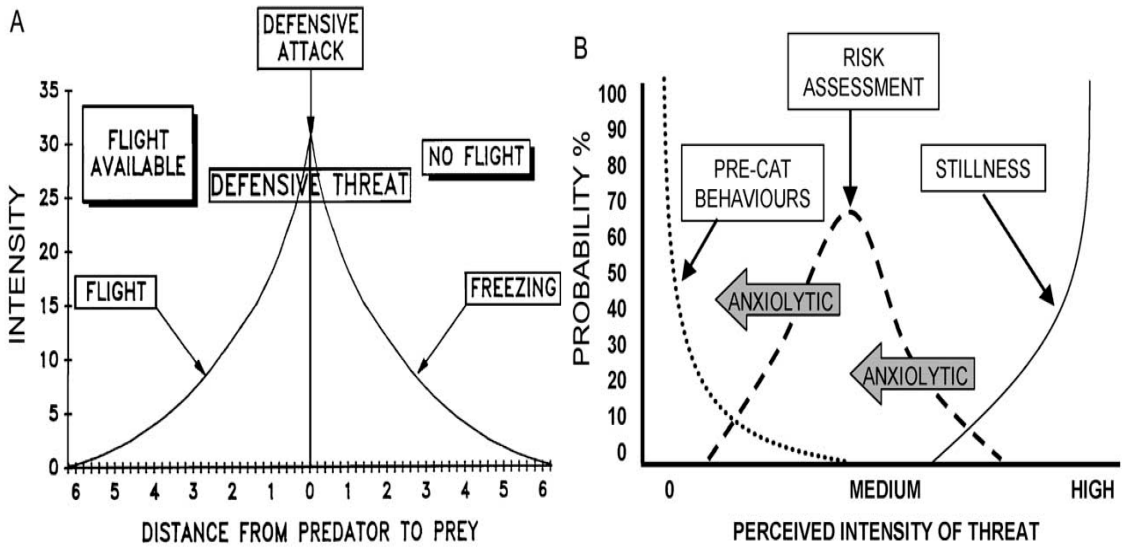


Figure 8 ***The relationship between defensive distance and behaviour. A: For defensive avoidance, B: For defensive approach. The grey arrows represent a fixed change in defensive distance produced by anxiolytic drugs both increasing and decreasing risk assessment behaviour depending on the initial defensive distance.***
***(Adapted from McNaughton, 2004)* (66)**

The neural systems controlling defence is based on two behavioural dimensions: 'defensive distance' and 'defensive direction'. Defensive direction is a categorical dimension with avoidance of threat corresponding to fear and approach to threat corresponding to anxiety. Depending on the gravity of threat assessment, and the perceived distance to the threat, different defence strategies will be enacted, with the appropriate accompanying degree of neural-activation deemed necessary to deal with the impending need. These two psychological dimensions are mapped to underlying neural dimensions:

> *"Defensive distance is mapped to neural level, with the shortest defensive distances involving the lowest neural level (periaqueductal grey (PAG)) and the largest defensive distances the highest neural level (prefrontal cortex). Defensive direction is mapped to separate parallel streams that run across these levels. A significant departure from prior models is the proposal that both fear and anxiety are represented at all levels."* (66)

In this schema, different behavioural and autonomic responses relate to "defensive distance" and in particular whether a threat is avoidable or unavoidable. (Figure 9)

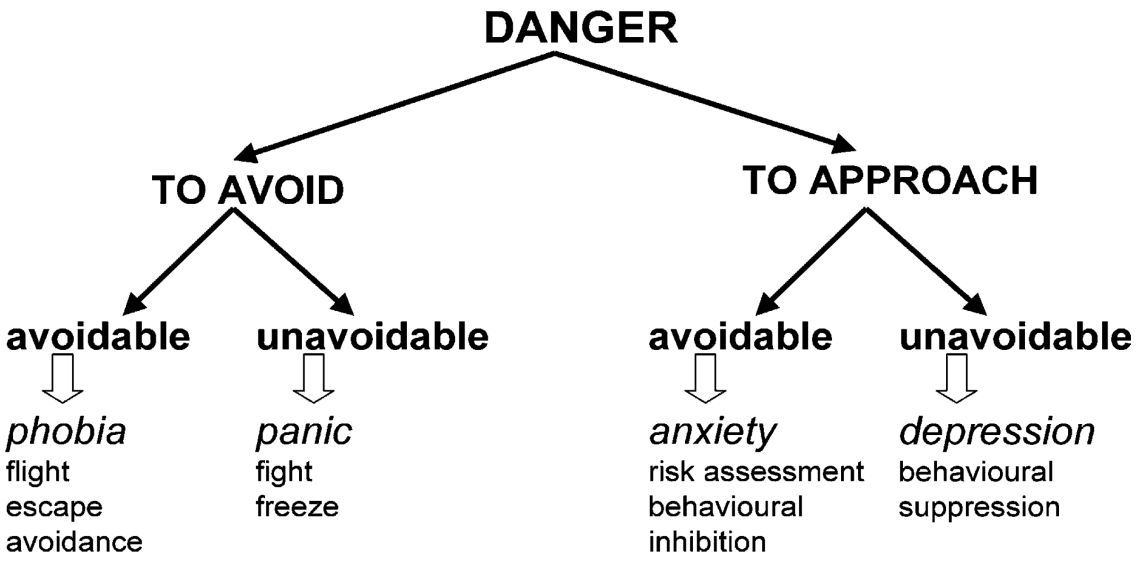


Figure 9 ***Categories of emotion and defensive response derived from defensive direction (avoid or approach the danger) and avoidability of the threat.***
(Adapted from McNaughton, 2004) (66)

Hence, 'simple ' avoidable threats will be dealt with by straightforward behavioural responses, and only lower neural (i.e. PAG) activation. More complex inescapable chronic threats will involve the higher neural circuits (i.e. prefrontal cortex) where learnt/conditioned factors based on personality and prior experience play an ever increasing role, resulting in a more complicated portfolio of differing behaviours, associated with varying degrees of accompanying neuronal arousal. (Figure 10)

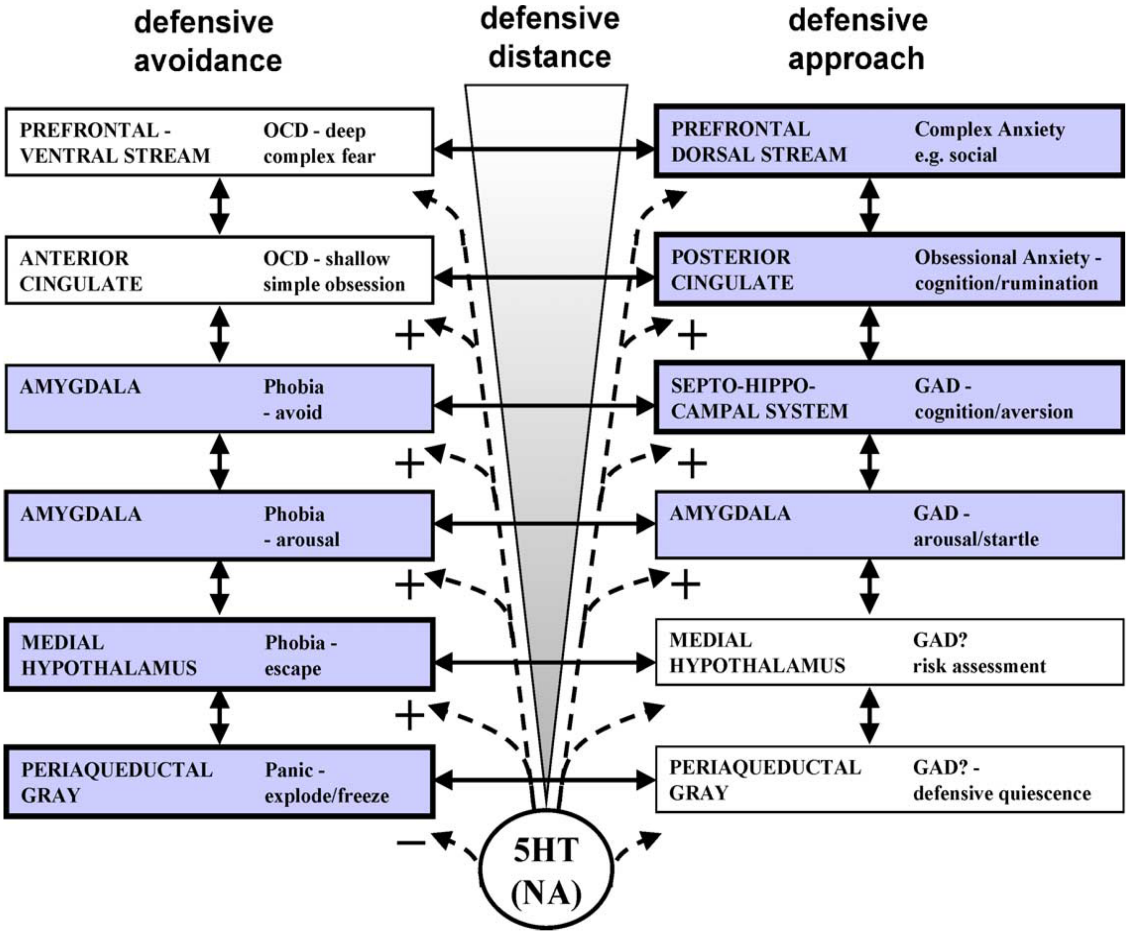


Figure 10 ***The two-dimensional defence system. On either side are defensive avoidance and defensive approach respectively (a categorical dimension). Each is divided, down the page, into a number of hierarchical levels. These are ordered from high to low (top to bottom) both with respect to neural level (and cytoarchitectonic complexity) and to functional level. Each level is associated with specific classes of behaviour and so symptom and syndrome. Syndromes are associated with hyper-reactivity of a structure and symptoms with high activity. Given the interconnections within the system (and effects of e.g. conditioning) symptoms will not be a good guide to syndromes. [OCD: obsessive-compulsive disorder, GAD: generalised-anxiety disorder]***
(Adapted from McNaughton, 2004) **(66)**

Finally, they thus relate the two types of defence response and defensive distance to a hierarchical neural (defence) response activation system, which is associated with specific classes of behaviour, neural involvement and resulting ANS activation, which is where we turn to next.

1.6.5 Pain, the Autonomic Nervous System, and Psychology

Emotions during acute stress (e.g. during painful procedures) are associated with high heart rate and high pitch vocalisations and cries in animals and infants. When considering ANS responses in this context, both characteristics are determined by a withdrawal of vagal efferent outflow originating in the nucleus ambiguous (NA). This is because the branch of the vagus originating in the (right) NA is closely linked to the rapid expression and regulation of emotional states. (100) Cardiac vagal tone (CVT) can therefore be used as an *"index of central-peripheral neural feedback and CNS-ANS integration"* (107) and in addition to pain reactivity has been linked to psychological trait differences such as temperament, emotionality and the process of interoception. (70, 108)

Life events, stress and physical strain are potential factors that interact with the "emotional motor system" (EMS), and represent the "*highest neural level*" as referred to in the previous section by Gray and McNaughton. This is made up by the insula, pre-frontal-and-anterior cingulate cortices and the amygdala, that has a regulatory effect on the ANS, via the ventral vagal nuclei within the right NA, to modulate and govern an individual's visceral pain sensitivity. (70) Stress, either exteroceptive (psychological/environmental) or interoceptive (somatic/visceral) activates the EMS and the resulting autonomic and neuroendocrine responses and so doing modulates the pain response sensitivity. (109) Dysfunction of these systems is hence relevant as modulators in the pathophysiology of the visceral pain hypersensitivity seen in FGID. (110) (Figure 11)

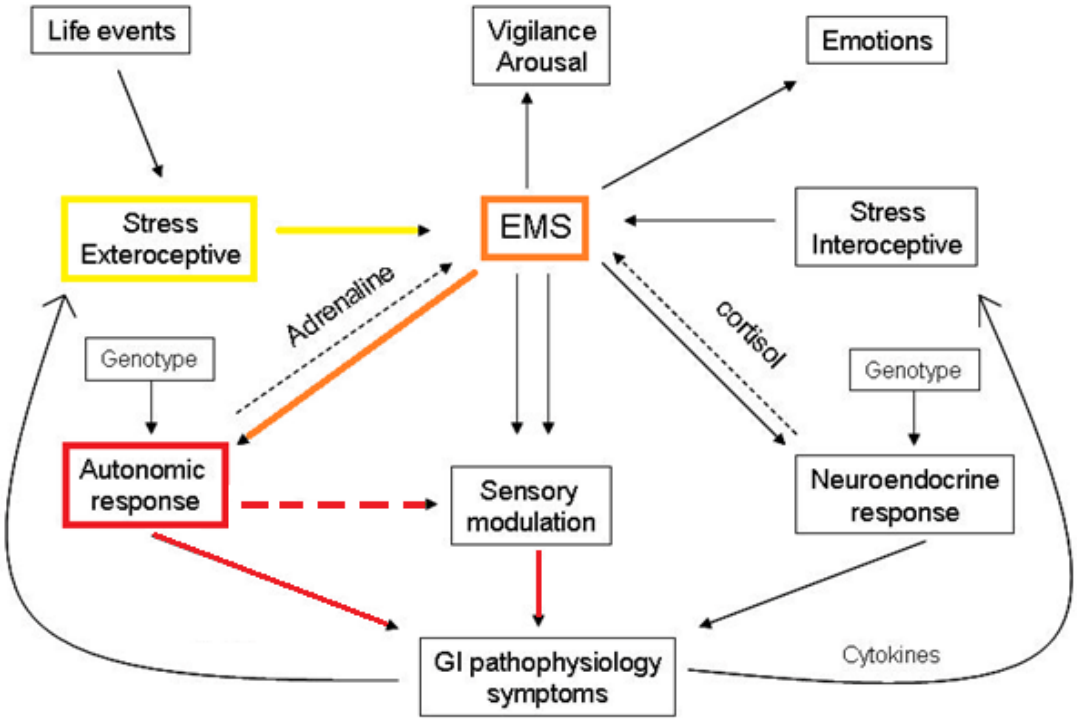


Figure 11 ***Shows the potential factors that interact with the Emotional Motor System (EMS) and has an effect on the ANS that governs an individual's visceral pain sensitivity. (Adapted from Drossman, 2004)*** **(109)**

Traditionally pain researchers exploring the neuroendocrinal defence responses have emphasised the "*fight-n-flight*" pattern and its associated behavioural activation, sensitisation and sympathetic nervous system (SNS) reactivity. (60, 111) Recent research has led to a growing appreciation and understanding of the hierarchical superiority of the inhibitory control over behavioural activation systems brought about by the SNS. This includes inhibition or conversely disinhibition especially from pre-frontal cortices, together with parasympathetic nervous system (PNS) activity in a broad variety of responses ranging from the "*freeze*" defence response to and also including the 'bonding' or affiliative "*mend-n-befriend*" behaviours. (112-117) Describing this arm of the neuroendocrinal defence responses has led to a wide range of overlapping behaviours and a somewhat confusing nomenclature that includes freeze, vigilance, quiescence, cautious-approach, tonic-

immobility, fright, faint, passiveness, submissiveness and mindful coping. (100, 118)

1.8 The Psychosocial determinants of Pain and Attachment Theory

John Bowlby first used the term "attachment" to describe the affective bond that develops between an infant and a primary caregiver.[5] (119) He believed that the "attachment behavioural system" was one of four behavioural systems that are innate and evolutionarily functions to assure survival of the species. The quality of attachment evolves over time as the infant interacts with his/her caregivers, and is then internalised as implicit memories, regulating limbic reactivity. (Figure 12)

Figure 12 ***Shows four behavioural systems of attachment that are innate and evolutionarily function to assure survival of the species.***
(Adapted from Bowlby, 1958)**(119)**

[5] Bowlby's departure from the traditional psychoanalytic theory at the time was considered heretical, and was ostracised by his peers for many years to come. It wasn't until after his death in 1990 that the British analytic community issued a formal apology to his family.

Attachment relationships evolve over the first two years of life and beyond, but most importantly these early attachment relationships overlap with a time of significant neurological development of the brain. (120) Experiences in those early relationships encode in the neural circuitry of our brains by 12-18 months of age, and because they are entirely in implicit memories, they are outside of conscious awareness, and hence are "known but not remembered". Unconsciously they form the patterns of attachment, which become the "rules", templates and schemas, for relating that operate lifelong, as the 'givens' of our interpersonal-relational lives.

This is of significance as it has now been established that experience shapes the brain by the following sequence: Any experience causes neurons in our brains to fire. Repeated experiences cause neurons to fire repeatedly. Neurons that "*fire together wire together*,"[6] (121) strengthening neural connections through long-term potentiation. Strong neural connections become neural pathways and neural networks, which activate genes, which then lead to the production of proteins that enable the formation of new synaptic connections. (48, 122) This experience-triggered neural firing is how all-neural pathways become patterns of response, and how all structures of the brain mature. This is how all patterns of attachment are laid down in the brain; it is also how they can change. It is likely, though not yet directly proven in human studies, that the experiences within attachment relationships shape the emerging neural circuitry of the child's developing brain. This shaping process, for example, enables parent-child interactions to shape the genetically programmed maturation of the brain to alter the ways in

[6] Hebbian theory: a scientific theory in biological neuroscience, which explains the adaptation of neurons in the brain during the learning process. It describes a basic mechanism for synaptic plasticity wherein an increase in synaptic efficacy arises from the presynaptic cell's *repeated* and *persistent* stimulation of the postsynaptic cell. Introduced by Donald Hebb, 1949.

which such fundamental processes as emotion regulation, response to stress, autobiographical memory and even theory of mind, which is central in assessing interpersonal intention and threat, develop.

Daniel Siegel, expands on this and gives a neuro-scientific understanding to our internal subjective and interpersonal social lives, as:

> *"…these findings show how the brain has evolved as a social organ of the body. Mammals are social creatures, with limbic structures that appear to serve the dual purpose of attuning to the social environment while regulating the internal state of the body. The limbic circuits help us understand the mammalian trait of needing the presence of caregivers to help regulate the physiology of the young infant."(123)*

As that infant mammal grows, its ability to regulate its own physiology in a balanced manner will develop a more autonomous capacity. Studies of maternal deprivation in rats have shown that permanent alterations in the behavioural and physiological response to stress occur and impact the social functioning and regulation of the maturing animal. (124, 125) Bremner states:

> *"While infants can be seen in general as being adaptive, research clearly shows how early adverse experience can have negative effects on growing brains that have persistent effects on functioning."* (126)

When differentiation is combined with integration, the complex homeostatic system of the brain is able to achieve highly adaptive, flexible and stable states of functioning. Such a state can be proposed to be synonymous with more mental resilience. (127) In this way secure

attachment relationships may promote well-being by supporting the integrative capacities of the child's developing brain. (128) As it is well known that "*the child is father to the man,*"[7] these integrative capacities associated with attachment and physiological activation become stable trait phenomena affecting stress responses on a global level, that influence regulatory processes throughout life. (127, 129) This research is still at a very early stage however, and further work is needed to assess the processes associated with attachment and physiological activation, and its eventual longitudinal impact on chronic pain conditions.

1.9 The Neuronal Regulation of Pain

1.9.1 Pain Regulation by the ANS

Benarroch (100, 159) and Cortelli (160) have recently extensively reviewed neuroanatomical pain regulation of the ANS for both acute and chronic pain. They observed that nociceptive processes and the ANS interact at the level of the periphery, spinal cord, brainstem, and forebrain. (91, 130) Spinal and visceral afferents provide converging ascending afferent information to spinothalamic neurons in the dorsal horn and to neurons of the nucleus of the solitary tract (NTS) and parabrachial nuclei. As previously discussed above, these structures project to sub-cortical areas involved in reflexive, homeostatic, and psycho-behavioural control of autonomic outflow, endocrine function, and nociception.

The PNS and SNS have mainly reciprocal activities in the modulation of pain. Whilst the PNS (vagus) is broadly antinociceptive (131); the SNS is broadly pronociceptive. (132, 133) The implication of this is that a

[7] William Wordsworth, 1888

balance between sympathetic and parasympathetic activities is required for normal pain perception. It is however only a "rule of thumb" as there remain some contradicting findings that are not fully understood, as for example the co-activation of the ANS during pain, mediated via the NTS, which includes the possibility of PNS facilitation during pain regulation. (134)

1.9.2 Co-activation in pain and the NTS

Boscan and Paton (134-137) experimentally confirmed while studying the rat's autonomic control of pain, that there were unanticipated complex possibilities of non-reciprocal, co-activation of the ANS. When they applied noxious stimulation to the forelimb of rats, it evoked burst discharges not only in the inferior cardiac and lumbar sympathetic nerves, but surprisingly also in the cardiac branch of the vagal nerve. As the usual response was a tachycardia, the increased vagal activation was puzzling and unexpected, as it suggested that during nociception both sympathetic and parasympathetic cardiac outflows were co-activated. This led them to propose a novel 'paradoxical role' for the PNS, which mediates tachycardia during nociception that is integrated by the NTS. (Figure 13)(134)

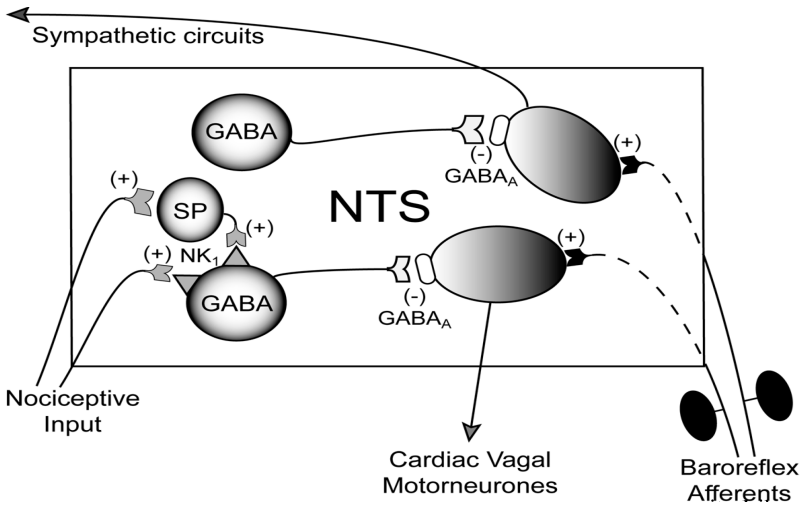


Figure 13 ***Showing the proposed NTS integration of nociceptive and baroreflex afferent input with both sympathetic and 'paradoxical' cardiac vagal motor-neurone output. (Adapted from Pickering, 2003)*****(134)**

Because of phenomena like the above mentioned 'unanticipated complex non-reciprocal ANS co-activation', attempts to come to a more coherent understanding of the finer nuances in ANS interaction, more sophisticated models of ANS regulation need to be mentioned.

1.9.3 The "Dynamic Systems Approach" of ANS regulation

Recordati, emphasising the ANS as a key player in any homeostatic functional neural network, stated:

> *"The autonomic nervous system as a whole may be viewed as a dissipative structure progressively assembled in the course of evolution, plastically and rhythmically interfaced between forebrain, internal and external environments, to regulate energy, matter and information exchanges"* (138)

Hence in order to consider the ANS as a single functional unit, the interactive function of components of the ANS was conceptualised by Berntson *et al.* as being 'shaped' by "Autonomic Determinism"; which proposes that,

> *"…the multiple modes of autonomic control do not lie along a single continuum extending from parasympathetic to sympathetic dominance but rather distributed within a 2-dimensional space".* (139)

This concept of '2-D autonomic space' becomes even more complicated as levels are included of reactive lability as a function of the direction of movement (mode of control) within '3-D autonomic space'. (Figure 14) These fluctuations in ANS regulation need now to be correlated and time locked with specific psychophysiological events in laboratory conditions, in order to become more practically applicable in aiding deeper understanding of syndromes seen clinically.

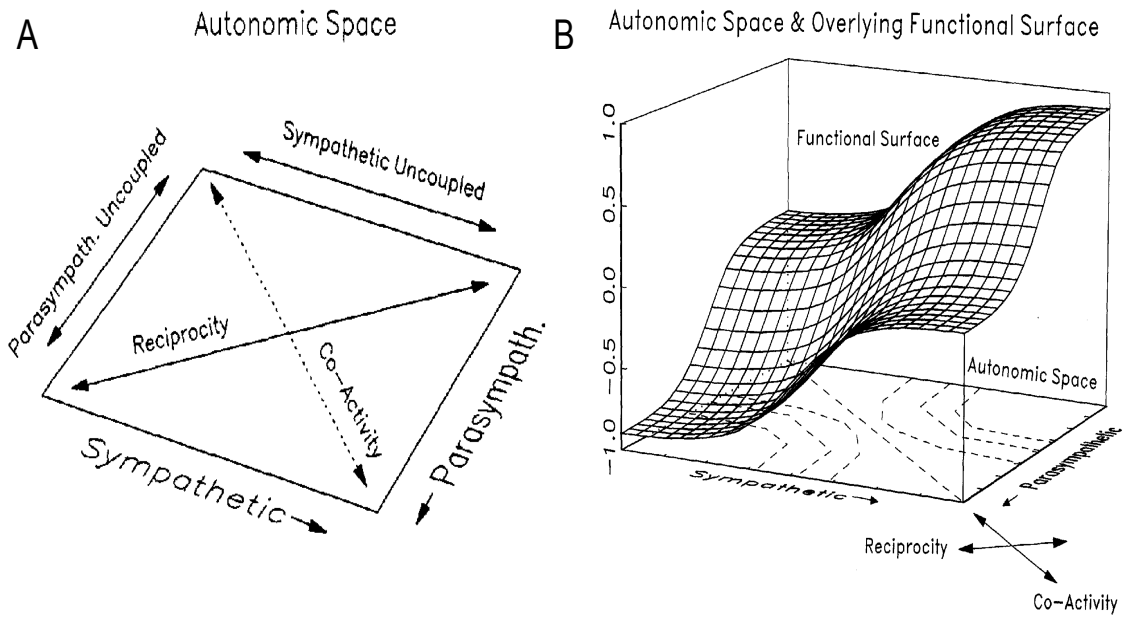


Figure 14 (A) 2-dimensional model of "autonomic space", (B) 3-dimensional model of autonomic space and its associated functional surface. The functional surface represents the operational state of the target organ, expressed in relative units. The axes dimensions are in decline units of functional activation. Dotted lines represent iso-functional contour lines projected on the autonomic space, illustrating loci within autonomic space that have equivalent functional outputs. The functional surface of autonomic space represents the operational state of the target organ, and depicts the gradients [sum of the partial derivatives] of the functional surface across autonomic space. Variations in the surface amplitude in these figures illustrate the instantaneous changes in organ state associated with the indicated movement from any point in autonomic space. (Adapted from Berntson, 1991) (139)

1.9.4 The "Polyvagal Theory" of PNS regulation

Recordati, quoted above with regard to the 'dynamic systems approach' to ANS regulation, continues to expand on the key-role of the PNS in maintaining stability in metabolic homeostatic regulation:

> *"…for spontaneously stable states to occur, slowing of the metabolic rate, withdrawal of the sympathetic drive and reinforcement of the vagal tone to the heart and circulation are required, thus confirming that the parasympathetic division of the autonomic nervous system is the main controller of homeostasis"* (138)

In order to comprehend how this is achieved, no other body of knowledge has been as influential in shaping our understanding, and influencing research in this field, as Porges's 'Polyvagal Theory'. (108, 118) It has emerged from a phylogenetic (and ontogenetic) study of physiological self-regulation with emphasis on the comparative functional anatomy of the autonomic nervous system and of the vagus in particular. (140) Figure 15 shows the results of this phylogenetic comparison of vertebrates.

Neural regulation of the heart as a function of vertebrate phylogeny

Group	CHM	DVC	SNS	AD/m	VVC
Jawless fish	X+	(X+)			
Cartilaginous fish	X+	X−			
Bony fish	X+	X−	X+		
Amphibians	X+	X−	X+		
Reptiles	X+	X−	X+	X+	
Mammals	X+	X−	X+	X+	X−

Abbreviations: CHM, chromaffin tissue; DVC, dorsal vagal complex with vagal efferent pathways originating in the dorsal motor nucleus of the vagus and vagal afferents terminating in the nucleus of the solitary tract (NTS); SNS, spinal sympathetic nervous system; AD/m, adrenal medulla; and VVC, ventral vagal complex with efferent pathways originating in the nucleus ambiguus that regulate visceral structures (heart, bronchi, thymus) and striated muscles via special visceral efferents and afferents via the solitary tract, trigeminal and facial nerve. X+ indicates a cardioexcitatory influence (e.g. increases in heart rate). X− indicates a cardioinhibitory influence (e.g. decreases in heart rate).

Figure 15 ***Showing the phylogenetic comparison of Cardio Vagal Control (CVC) (Adapted from Porges, 1991)*** **(70)**

The vagus nerve emerges from or converges onto four nuclei of the medulla. About 90% of the vagus is sensory and is represented by the solitary and spino-trigeminal nuclei. The nucleus of the solitary tract (NTS) receives afferent taste information and primary afferents from visceral organs, which carry information from the thoracic, oesophageal and abdominal viscera; including afferents from the aortic body and arch. It's relevance is not only due to its role in oesophageal sensory conduction, but also in forming the afferent part of the cardiac/respiratory reflex which is a central part of PNS control. (see Figure 44, page 120)

The remaining 10% of the vagus is motoric and has two brainstem nuclei. (Figure 16) The nucleus ambiguous (NA) gives rise to myelinated fast-effector neurones, which innervate the heart; larynx and upper gut,

whilst the dorsal motor vagal nucleus (DMNX) gives rise to unmyelinated slow effector neurones innervating the heart and lower gut. Extreme DMNX output results in a profound bradycardia, occurring in immobilisation behaviours such as death feigning and passive avoidance in reptiles and lower mammals. It is a very primitive defence response and potentially fatal in higher mammals (possibly associated with 'Sudden unexpected death syndrome' (SUNDS)(141), and 'Sudden infant death syndrome' (SIDS) (142)), with high oxygen needy metabolic rates, although adaptive for organisms with low metabolic rates. (70) Recent research findings indicate however that more subtle activation of the DMNX occurs throughout day-to-day ANS regulation in environments of potential threat, (117) as posited by Gray and McNaughton with their hierarchical neural (defence) response activation system. (64-66, 104-106) (See also table 9, chapter 4, page 201, for stages of ANS activation to stress.)

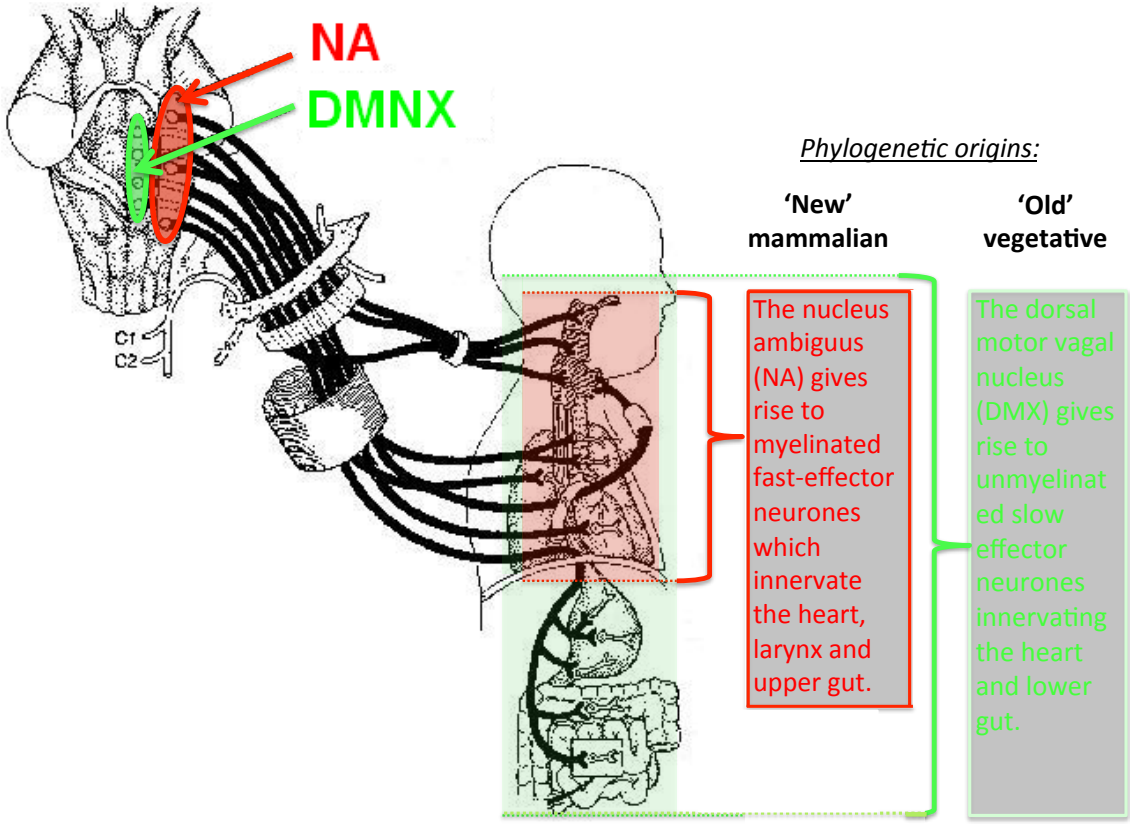


Figure 16 ***Showing the two vagal brainstem motor nuclei and their extension in the viscera.*** ***(Based on Porges, 2009)*** **(117)**

In contrast the NA output (CVC_{NA}) allows for subtler beat-to-beat heart rate modulation – increased CVC_{NA} causes milder slowing of the heart rate which may be involved in behavioural inhibition and bonding whilst withdrawal of CVC_{NA} reduces the external constraint on the intrinsic sino-atrial node (SA) automaticity and therefore a faster heart-rate ensues to facilitate behavioural activation. This has been described as the "vagal brake"(143), which Recordati describes as the most efficient and neuro-chemically "cost-effective" way of maintaining homeostatic control of the heart and circulation. (138)

1.9.5 Adrenergic modulation of pain

This occurs in the periphery, spinal and supra-spinal sites. In the periphery there is little baseline adrenergic modulation of pain. However, following tissue injury, nor-adrenalin induces novel noradrenergic receptors. It also induces sympathetic nerve sprouting and alters the ionic channel properties of primary afferent nociceptors (N-type Ca2+ channels) and all of these are pronociceptive activities leading to hyperalgesia. (144)

1.9.6 Spinal adrenergic modulation of pain

They are nearly all inhibitory. Pre-synaptic inhibition of the primary afferent nociceptor terminals in the dorsal horn of the spinal cord is mediated through alpha-adrenoreceptors. There is also evidence of a direct adrenergic action on pain relay interneurones through post-synaptic inhibition mediated by alpha2-adrenoreceptors. Alpha1-Adrenergic mediated activation of inhibitory interneurones is another mechanism of spinal adrenergic antinociception. (144) They are mostly peptidergic and contain first class neurones, which express peptide neurotransmitters such as substance P and CGRP (calcitonin gene related peptide). (Figure 17) (145)

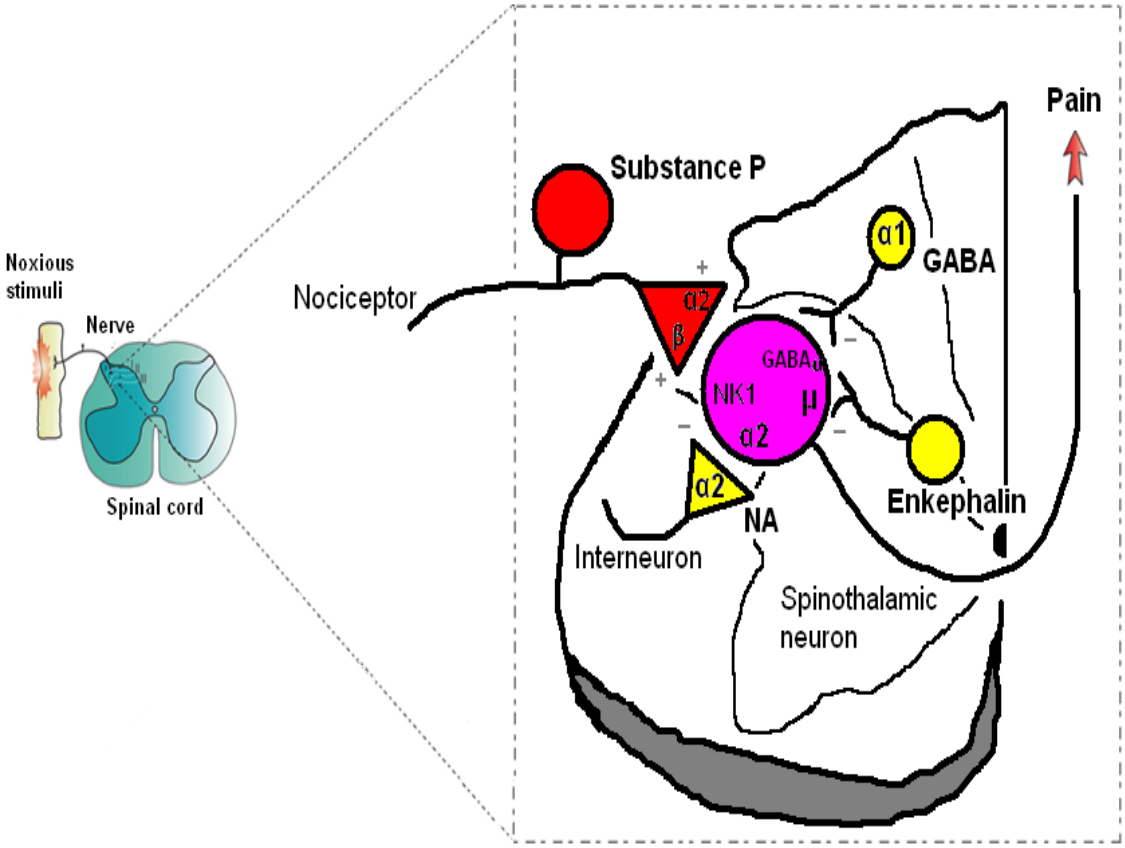


Figure 17 *Showing the spinal α1 and α2- adrenergic synapses in the dorsal horn. Post-synaptic inhibition mediated by alpha2-adrenoreceptors, activation of inhibitory interneurones, which is another mechanism of spinal adrenergic antinociception, which express peptide neurotransmitters such as substance P, and calcitonin gene related peptide (CGRP).* *(Adapted from Pertovaara, 2006)* **(144)**

1.9.7 Supra-spinal adrenergic modulation of pain

The visceral projection from the spinal cord to subcortical and cortical structures consists of several pathways. The spinothalamic tract terminates in the medial and posterior thalamus. Thalamocortical fibres then project to the somatosensory cortex. The spinoreticular tract terminates in the reticular formation in the brainstem. The reticulothalamic tract projects from the dorsal and caudal medullary reticular formation to the medial thalamus. The spinomesencephalic tract projects to various regions in the brain stem, including the periaqueductal grey, locus coeruleus, and dorsal reticular nucleus in the

medulla. Thalamocortical projections from the medial thalamus project to the cingulate cortex and insula, which are involved in processing noxious visceral and somatic information. The brain regions innervated by these pathways that respond to painful visceral stimuli include the thalamus, insula, amygdala and anterior cingulate cortex (ACC). The ACC is comprised of two components, the perigenual ACC (pACC) involved in affect and midcingulate cortex (MCC) with behavioural response modification. (146)(Figure 18)

Supra-spinal adrenergic modulation of pain varies depending on the site of activity, the type of adrenoceptors, the duration and pathophysiology of the pain. The general observation is that at baseline conditions there is little adrenergic effect on pain perception. However, during sustained pain there is supra-spinal noradrenergic feedback inhibition of pain. (144) In the brainstem, the ventrolateral medullar oblongata, brainstem reticular formation, parabrachial nucleus and periaquaductal grey matter all receive ascending nociceptive afferents. (Figure 18) The rostral ventrolateral medullar oblongata is the main area where pre-sympathetic vasomotor neurones are situated together with other sympathetic driver neurones. (147) They are arranged here in organotopic manner and also occur in the dorsal vagal complex, in the bulbar reticular formation, in the ventrolateral pons, and in the Locus Coeruleus and others.

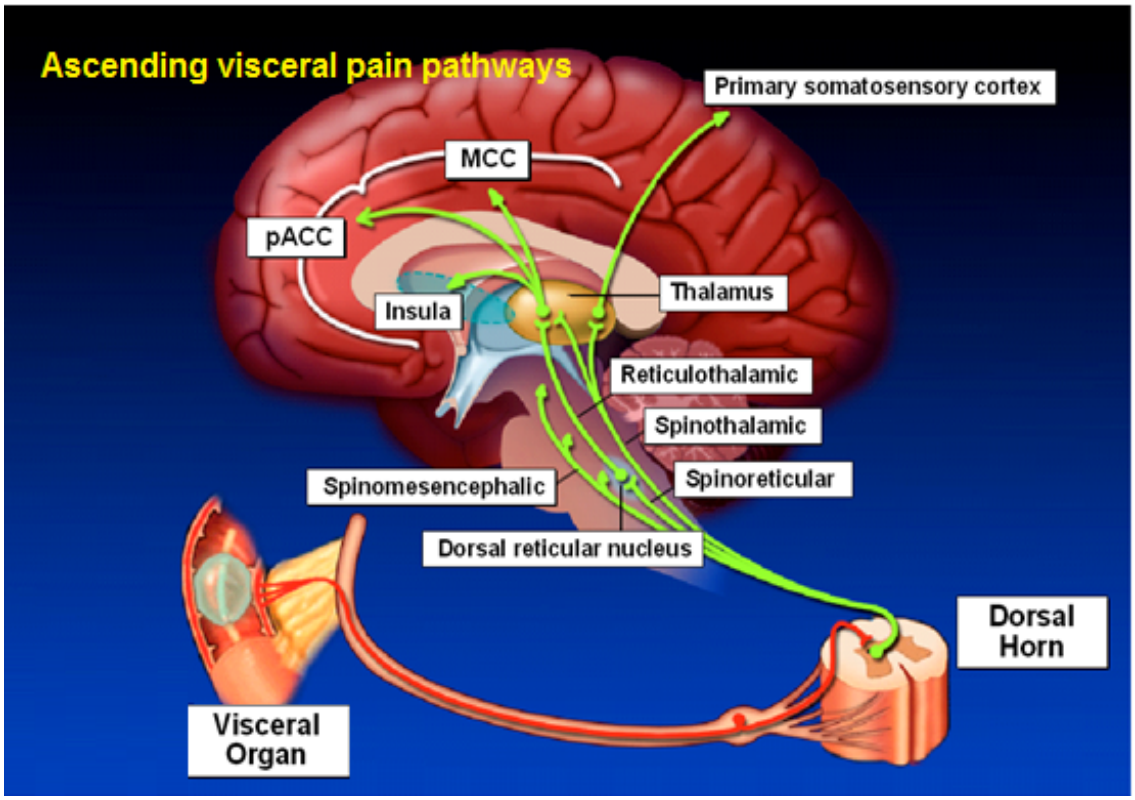


Figure 18 ***Shows the principal visceral projections from the spinal cord to subcortical and cortical structures (green lines). The spinothalamic tract terminates in the medial and posterior thalamus. Thalamocortical fibres then project to the somatosensory cortex. The spinoreticular tract terminates in the reticular formation in the brainstem. The reticulothalamic tract projects from the dorsal and caudal medullary reticular formation to the medial thalamus. The spinomesencephalic tract projects to various regions in the brain stem, including the periaqueductal grey, locus coeruleus, and dorsal reticular nucleus in the medulla. Thalamocortical projections from the medial thalamus project to the cingulate cortex and insula which are involved in processing noxious visceral and somatic information. The brain regions innervated by these pathways that respond to painful visceral stimuli include the thalamus, insula, amygdala and anterior cingulate cortex (ACC). The ACC is comprised of two components, the perigenual ACC (pACC) involved in affect and midcingulate cortex (MCC) with behavioral response modification. Other pathways for transmission of noxious visceral stimuli (such as the dorsal column pathway) exist, but are not shown. (Adapted from Drossman, 2004)*** **(109)**

1.9.8 Cholinergic modulation of pain: In the periphery

Cholinergic efferent nerve fibres in the vagus are the main source of parasympathetic activity in most organs in the abdomen and the thorax. Vagotomy induces hyperalgesia in the area supplied by the severed part of the vagus nerve, which can be reversed by denervation of the sympathetic supply to the adrenal medulla. (148) These observations

suggest that in the modulation of gut pain, vagal parasympathetic activity is balanced by adrenergic activity. Moreover, alpha2-adrenergic antagonists can reduce vagotomy-induced hyperalgesia in the gut. (149)

1.9.9 Spinal cholinergic modulation

Cholinergic activity within the spinal cord modulates pain perception via M2-muscarinic receptors that also mediate parasympathetic activity in the heart, smooth muscle and lacrimal glands. It is postulated that activation of spinal M2-muscarinic receptors causes release of adrenal catecholamines and that the anti-inflammatory effects of the catecholamines reduce inflammatory pain. (150)

1.9.10 Supra-spinal modulation

The autonomic centres relevant to supra-spinal modulation of pain are discussed above. However, in adults the consistent pattern emerging is that higher resting blood pressures are associated with relative hypoalgesia probably mediated by reflex PNS activation via baroreceptor stimulation. (151)

1.9.11 The Autonomic Nervous System, pain and the viscera

The principal components of descending pain modulatory pathways which are activated in response to painful visceral stimulus are the Ponto-medullary networks, including the periaqueductal grey (PAG), rostral ventral medulla (RVM) and the raphe nuclei, which are modulated by inputs from the anterior cingulate cortex (ACC), amygdala, and other cortical regions. (Figure 19)

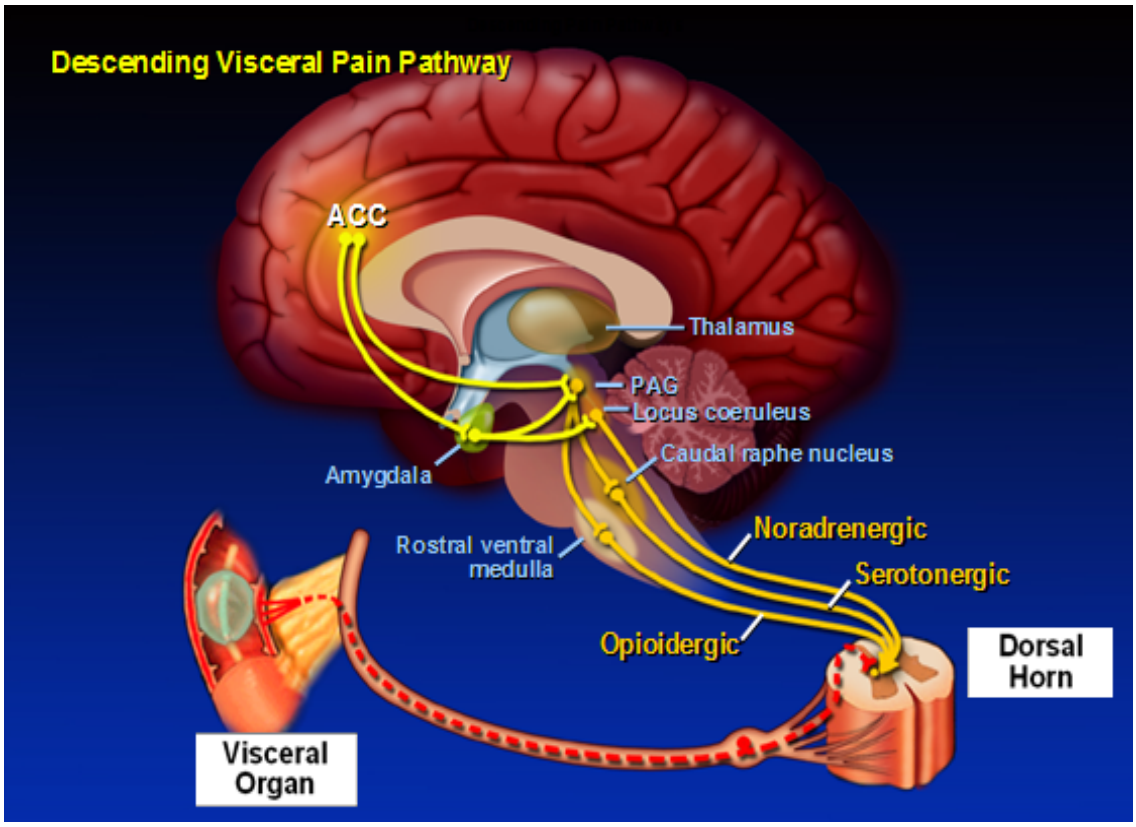


Figure 19 ***Shows the principal components of descending pain modulatory pathways (yellow lines), which are activated in response to a painful visceral stimulus such as noxious balloon distension of the colon. Ponto-medullary networks including the periaqueductal grey (PAG), rostral ventral medulla (RVM) and the raphe nuclei are modulated by inputs from the anterior cingulate cortex (ACC), amygdala, and other cortical regions. The major descending pain inhibitory pathways are mediated via the opioidergic, serotonergic and noradrenergic systems. These pathways modulate pain transmission at the level of the dorsal horn of the spinal cord.***

(Adapted from Drossman, 2004) **(109)**

The major descending pain inhibitory pathways are mediated via the opioidergic, serotonergic and noradrenergic systems. These pathways modulate pain transmission at the level of the dorsal horn of the spinal cord. There is mounting evidence to suggest that the interface between the gut lumen and sensorineural pathways is regulated closely by the ANS. (152) Increasing SNS activity has been shown to increase colonic sensitivity in healthy volunteers. (153) Enhanced sympathetic dominance to oesophageal acid infusion has been documented in patients with gastro-oesophageal reflux disease, (154) and reduced vagal activity has been reported in NCCP patients. (88, 155)

1.10 Modulation of pain by pharmacological modulation of the ANS

1.10.1 Pharmacological modulation of the SNS

There is evidence for both SNS and PNS influences on pain. Relationship between the SNS and pain has been repeatedly demonstrated in animals and humans and conditions such as 'reflex sympathetic dystrophy', sympathetically maintained pain' and the all-encompassing 'complex regional pain syndrome' which are well recognised sympathetically mediated clinical pain conditions. In these conditions sympathetic modulators such as alpha1 antagonist (phentolamine prazosin, terazosin) and ganglion blockers such as guanethidine are used as diagnostic tools. Efficacy of clonidine has been shown in these conditions as well as in numerous animal studies of sympathetically mediated pain. In animal studies, alpha 2 adrenergic agonists produce analgesia by actions in the periphery, supraspinal CNS, and in the spinal cord (156) Clonidine is believed to produce analgesia at the spinal level in part through stimulation of cholinergic interneurons in the spinal cord. Alpha 2 adrenergic agonists produce sedation and reduced blood pressure in addition to analgesia. Clonidine can be administered orally, transdermally (53) or spinally. When given orally it has 100% bioavailability and its peak concentration and maximal hypotensive effect is observed 1-3 hours later, and its half-life is 6-24 hours. Its analgesic effect is evident even when used as a single dose and has such been extensively used in anaesthesiology. In specific reference to the viscera, clonidine has effects on visceral pain perception in dyspeptic patients (107, 157) and in the colon of volunteers.

1.10.2 Pharmacological modulation of the PNS

This is effective in reducing pain in animal and human studies. Muscarinic agonists and antagonists (atropine) have been shown to

reduce and increase pain sensitivity respectively in rodents. This effect was associated with a corresponding increase or decrease in intraspinal release of acetylcholine depending on whether an agonist or antagonist was used. Furthermore there is evidence for the pro-algesic effects of atropine in humans. (158) During the 1990s, the discovery of the antinociceptive properties of the potent nAChR agonist epibatidine in rodents sparked interest in the analgesic potential of this class of compounds. A number of novel nAChR agonists with antinociceptive activity and improved safety profiles in preclinical models have now been identified, of these ABT-594 is the most advanced and is currently in Phase II clinical evaluation.

1.10 The human oesophagus

The main physiological function of the oesophagus is that of transporting nutrition and fluids to the rest of the digestive system. The human oesophagus is unique in its anatomical composition, as the proximal third is composed of striated muscle while the distal two thirds are composed of smooth muscle. (159) The proximal oesophagus has a dense spinal somatic-like innervation containing mostly myelinated visceral afferents. On the other hand the distal oesophagus contains mostly unmyelinated C-fibres with a comparatively less dense spinal innervation as would be found in other gut structures.

1.11 Sensory Innervation of the oesophagus

1.11.1 Sensory innervation at mucosal level

The alimentary canal is innervated by four populations of sensory neurons (Figure 20), - two intrinsic and two extrinsic.

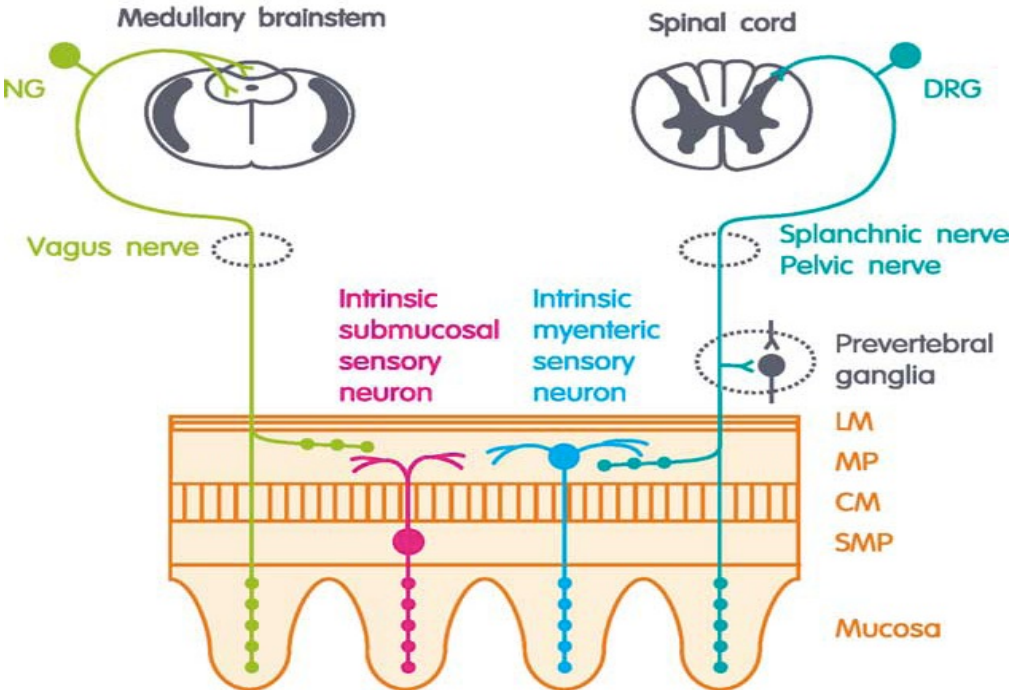


Figure 20 ***Innervation of the GI tract by intrinsic and extrinsic sensory neurons. The two populations of intrinsic primary afferent neurons originate in the submucosal plexus (SMP) and myenteric plexus (MP), respectively. The two populations of extrinsic sensory neurons are vagal afferents originating from the nodose ganglia (NG) and spinal afferents originating from the dorsal root ganglia (DRG). CM, circular muscle; LM, longitudinal muscle.*** ***(Adapted from Holzer, 2001)*** **(160)**

The populations of intrinsic primary afferent neurons (IPANs) have their cell bodies either in the myenteric plexus (Auerbach plexus) or in the submucosal plexus (Meissner plexus) and innervate both mucosal and muscular layers of the gut. (Figure 20 & 21) (146) Being part of the enteric nervous system (ENS), they comprise mucosal chemosensors, mucosal mechanosensors and muscular tension receptors. In addition, IPANs synapse with each other and in this way form self-reinforcing networks that issue outputs to interneurons, motor neurons, secretomotor neurons and vasodilator neurons. (161, 162)

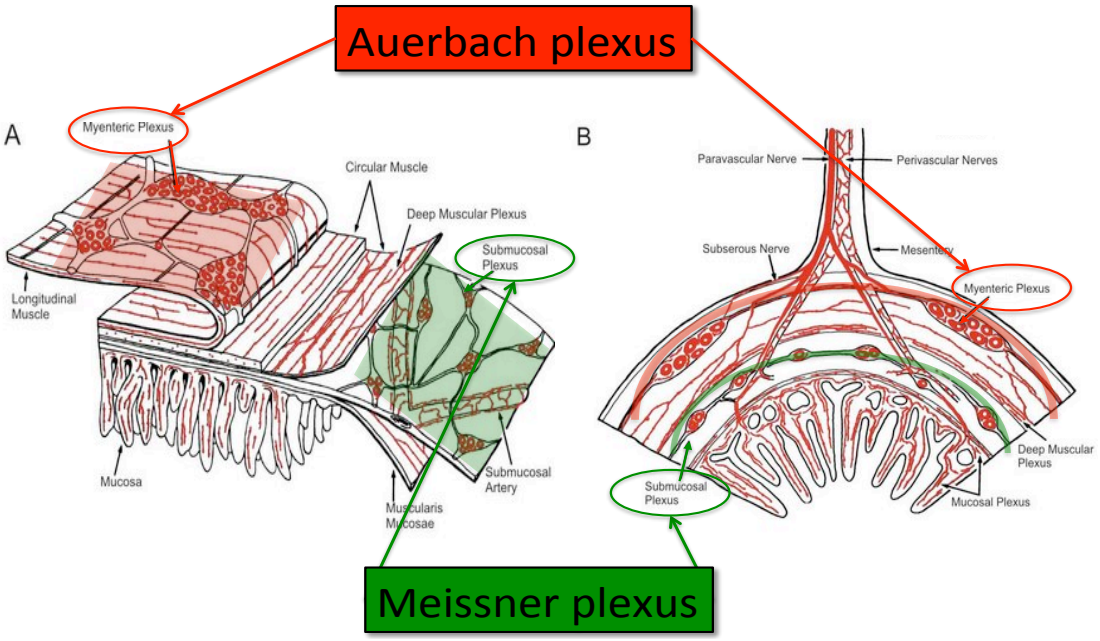


Figure 21 ***The arrangement of the enteric plexuses, depicted for the small intestine. A: appearance in separated layers. The myenteric plexus (Auerbach plexus), consisting of numerous ganglia and connecting nerve fibre bundles, lies between the longitudinal and circular muscle layers. A second ganglionated plexus (Meissner plexus) is in the submucosa. These plexuses provide nerve fibre plexuses in the muscle, in the mucosa and around arterioles. B: The enteric plexuses shown in a cross section of the intestine. (Adapted from Furness, 2007)*** **(163)**

The two populations of extrinsic sensory neurons (ESN) are vagal afferents with cell bodies in the nodose ganglia and spinal afferents with cell bodies in the dorsal root ganglia, and also contribute to the innervation of the circular muscle and the longitudinal muscle. Both the IPANs and the ESNs provide the ENS with the kind of information that is known as the "brain in the gut", and enables the requirements for autonomic control of digestion.

1.11.2 Sensory innervation at spinal level

As mentioned above the oesophagus receives innervation from both vagal and spinal nerves (Figure 22), however the majority of the oesophageal pain pathways are probably located in the spinal nerves. Dorsal root ganglia of cardiac and splanchnic nerves provide

craniocaudal innervation to the oesophagus. Afferent fibres then ascend centrally via spinothalamic tracts and dorsal columns to the thalamus and then on to the primary somatosensory cortex, insula, and anterior cingulate gyrus. (164) The spinal nerves enter the central nervous system through the dorsal root ganglion of the spinal cord from C1 to L2.

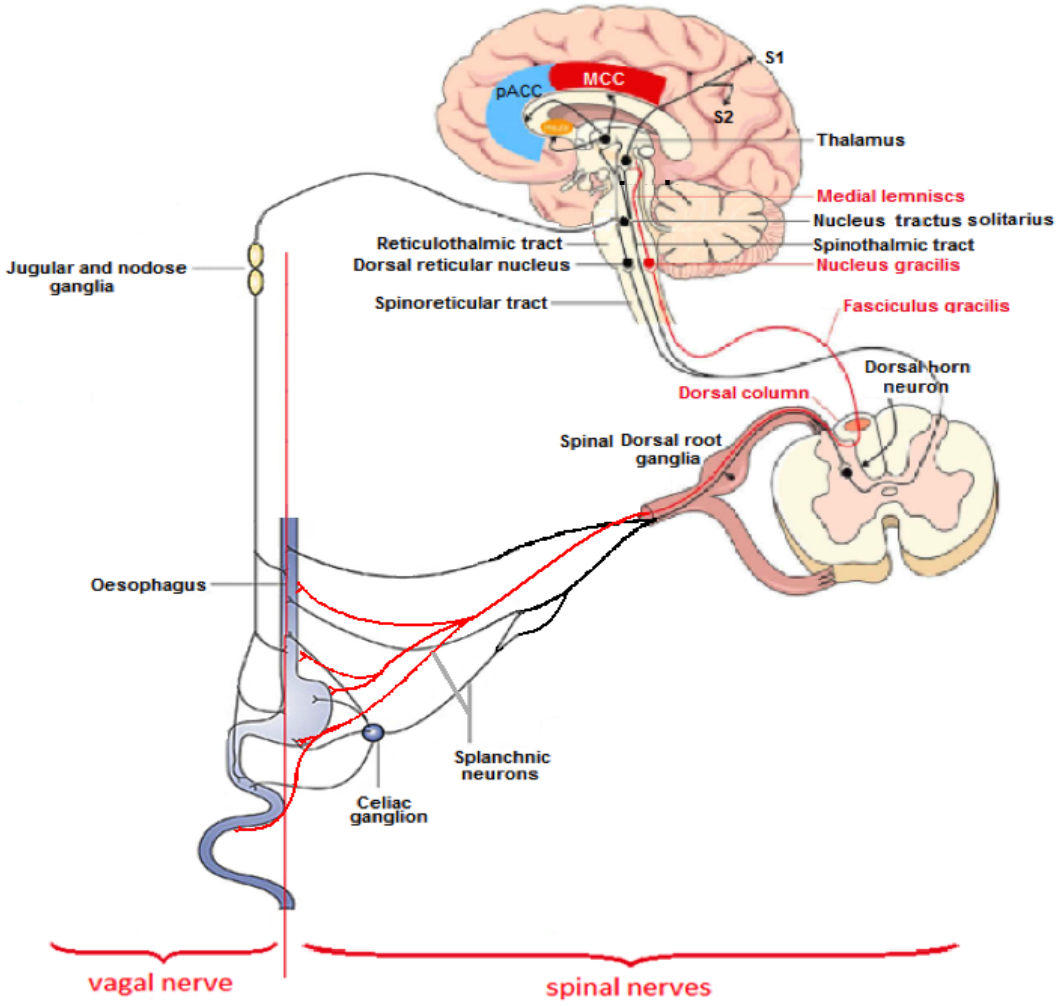


Figure 22 ***Schematic diagram of vagal and spinal nerve supply to the oesophagus. (Adapted from Sengupta, 1989)*** **(113)**

1.11.3 Sensory innervation at cranial nerve level

The vagal afferents travel with the main branch of the vagus nerve, primarily entering the central nervous system (CNS) via the nodose and

jugular ganglia and synapsing in the nucleus of the solitary tract. Most of these afferents (70-90%) are unmyelinated C fibres. (Figure 22) (164)

1.11.4 Sensory innervation at supra-spinal level

Positron electron tomography (PET) and functional magnetic resonance imaging (fMRI) have demonstrated that non-painful oesophageal sensation results in an increased regional blood flow bilaterally in the primary somatosensory cortex, bilaterally in the insular cortex, and frontal/parietal operculum. (165) Hobson *et al.* (166) found that painful oesophageal stimulation also activates the same regions but at an increased level as well as the involvement of the right anterior insular cortex and the anterior cingulate gyrus, further supporting and establishing the functional anatomical basis for the central component of pain processing in what is known as the "brain-gut axis".

When looking at the efferent functions, the division of the SNS and the PNS is true. However when one is looking at the afferent functional representation of the ANS, a division into the vagal and spinal afferent fibres is made. (167) (Figure 23) The vagal afferents nerves are composed mostly of unmyelinated C-fibres with few A-delta fibres terminating in bare nerve endings in each layer of the gut wall including serosa and mesentery. The spinal afferents have a greater role in visceral nociception. (168) Spinal afferents nerves can be further divided into splanchnic and pelvic afferents, which follow the actions of sympathetic and parasympathetic systems respectively. Spinal afferents may be divided into two nociceptive sensory receptor types, which innervate the viscera. (145)

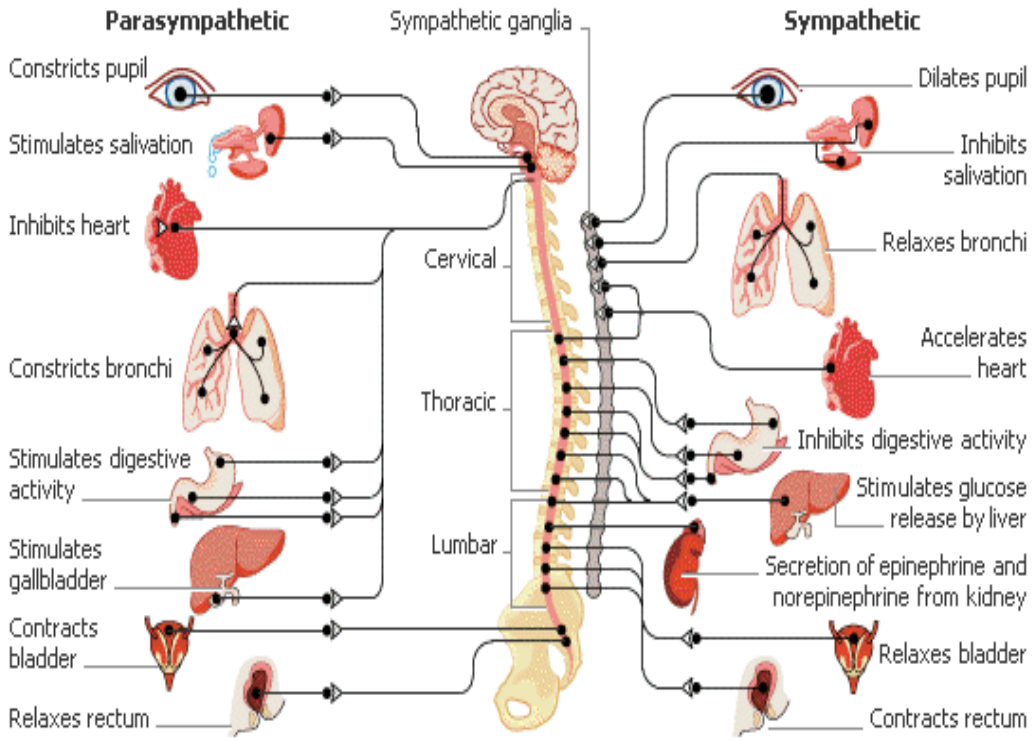


Figure 23 ***Showing the reciprocal actions of the para-and-sympathetic nervous systems. (Adapted from Wikipedia, 2013)*** **(169)**

Low threshold receptors have an encoding function (the relation between stimulus intensity to nerve activity) response, which is activated by innocuous and noxious stimuli. These receptors encode intensity and have been found in the oesophagus, heart, colon, bladder and testes. (48) High threshold mechano-receptors, these are activated entirely from a noxious stimulus to generate nerve activity. Silent nociceptors are a third group of receptors involved in nociceptor pain but they only become active after exposure to inflammatory mediators.

1.12 Oesophageal Pain Hypersensitivity

As mentioned above functional oesophageal pain or non-cardiac chest pain (which affects up to one third of those who undergo arteriograms for chest pain) (170) are also chronic functional symptoms which mimic

oesophageal disease yet do not have the same organic aetiology (171). As such they form part of the FGID group of disorders. VPH has been attributed as a factor underlying the pathophysiology in functional oesophageal disorders, as demonstrated in figure 24, (below) but the aetiology remains poorly understood. Gastro-oesophageal reflux disease (GORD) is common, with estimates of 20–44% of Western populations having symptoms of GORD at least once a month and 20% weekly.

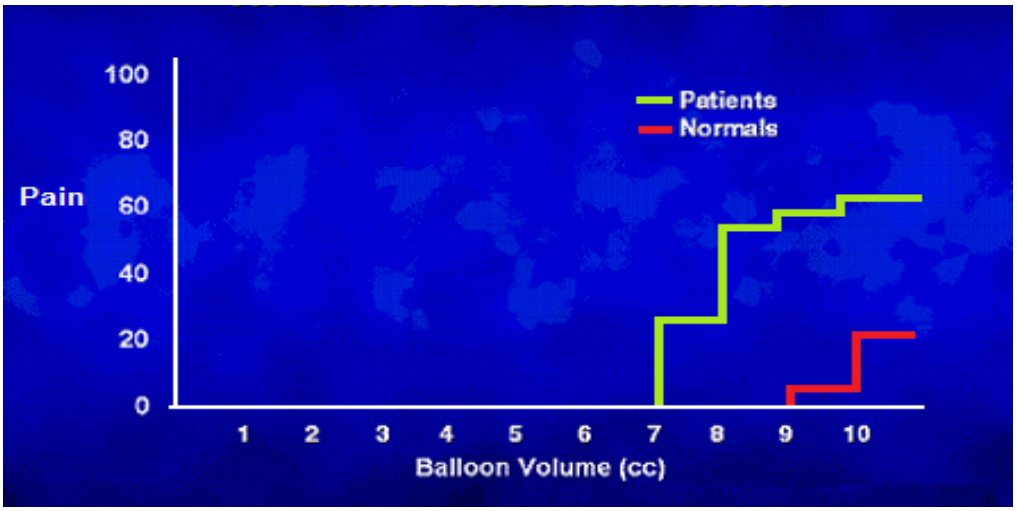


Figure 24 Oesophageal Pain with Balloon distension indicating clearly that there is hypersensitivity in the patient group. The graph indicates that their pain scores are much higher or lower volumes of balloon distension.
(Adapted from Paterson, Wang, & Vanner, 1995) (172)

In GORD there exists interplay between visceral hypersensitivity and acid exposure, leading to a spectrum of conditions (Figure 25, below), whereby moving from the right to the left there is an increase in acid exposure. Likewise moving from left to right there would be an increase in the role of hypersensitivity. In so doing one would then have a spectrum of conditions starting with erosive oesophagitis (EO) on the left side, where there is a clear emphasis on the acid exposure as the main aetiological factor and where ulceration or erosions are evident. On the right side the present evidence would support that the main aetiological

factor is VPH, presenting in conditions like functional heartburn (FH). This leaves us with the interaction of both acid exposure and visceral hypersensitivity in conditions like non-erosive reflux disease (NERD) in the middle.

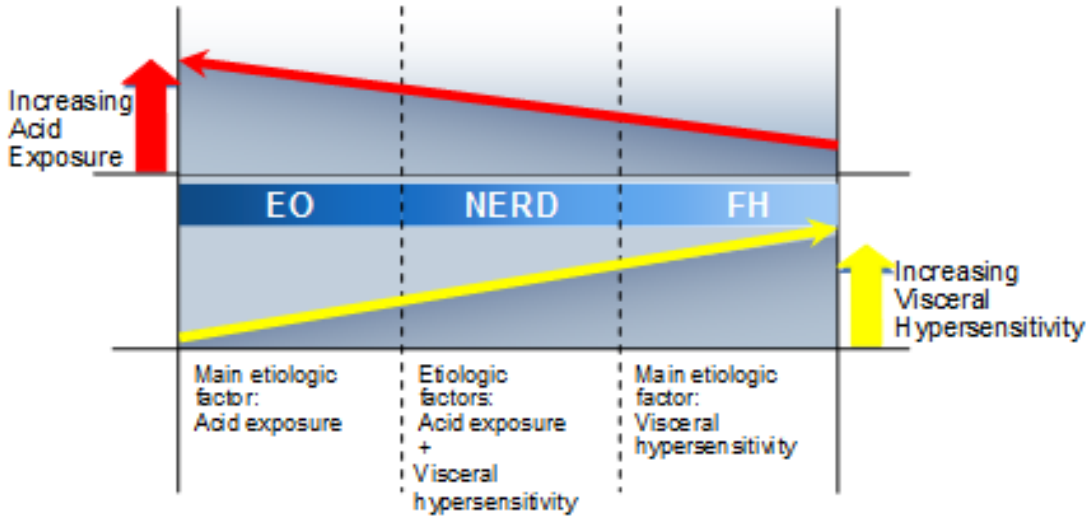


Figure 25 Shows the relationship that exists between acid exposure and visceral hypersensitivity in the aetiological role of gastro-oesophageal reflux disease (GORD). From left to right we have erosive oesophagitis (EO) where there is a high exposure to oesophageal acid. Then we have non-erosive reflux disease (NERD), where there is a combination of both acid exposure and visceral hypersensitivity and finally on the right-hand side we have functional heartburn (FH), where visceral hypersensitivity is clearly cited as the main aetiological cause. (Adapted from Knowles & Aziz, 2009)(167)

The proportion of patients with NERD is estimated to be between 50–70% of the GORD population. In these conditions it would be probable to expect that both the acid exposure and visceral hypersensitivity are contributing to the overall symptom profile observed, producing a perpetuating, mutually exacerbating course. What is presently still not clearly understood is the exact relationship that exists between the acid exposure and the visceral hypersensitivity and its interplay. This understanding would contribute significantly to the development of a more appropriate approach to treatment resulting in possible

substantially improved outcomes in the treatment resistant NERD patient group. Although VPH contributes to the clinical presentation in both EO & NERD, the fact that established responses to standard acid suppressive treatments are 20–30% lower in patients with NERD than those with EO, understanding the interaction between acid and VPH specifically in the NERD group remains a priority, and is thus the main concern of this thesis.

1.13 The need for a change in focus

Current management of pain in FGID involves the use of either antispasmodics or antidepressants. (173) Meta-analysis suggests that the former approach is no better than placebo while the latter approach produces global improvement without improving pain directly. (173) Pharmaceutical companies have invested heavily in the last two decades to develop the 'magic bullet' for managing pain in FGID, however their efforts have not met with success. Most medications developed on the basis of promising pre-clinical studies have either shown no effect or only a modest effect in clinical trials. (174) Part of the problem is that FGID is diagnosed on the basis of symptom-based criteria and hence there are considerable inter-individual differences in pathophysiology leading to heterogeneity in study populations. Furthermore, as discussed previously, there is a lack of disease biomarkers and good models of disease that can be used to test proof of mechanism for pharmacological preparations before large-scale clinical drug trials are performed. With the above considerations in mind what is proposed in this thesis is a mechanism-based approach to identify reasons for inter-individual differences in the development of VPH. This approach is based on a model of VPH previously developed and validated by several researchers in the field.

1.14 Work conducted on Human oesophageal model of peripheral and central sensitisation

To address the question whether inflammation/injury can induce PS and CS in the human GI tract, a model was developed which demonstrated that infusion of 0.15M hydrochloric acid into the healthy oesophagus reduced pain threshold reproducibly not only in the acid exposed region (peripheral sensitisation), but also in the adjacent unexposed region (central sensitisation). This effect was prolonged lasting up to 5 hours after 30 minutes of acid exposure (Figure 26) although a shorter 5 minute acid infusion also produced a transient hypersensitivity lasting for 2 hours. (175) Evidence of facilitated afferent pathways in the model has been obtained by a cortical evoked potential study demonstrating a decrease in latency and increase in amplitude of the response after acid infusion in comparison to saline.

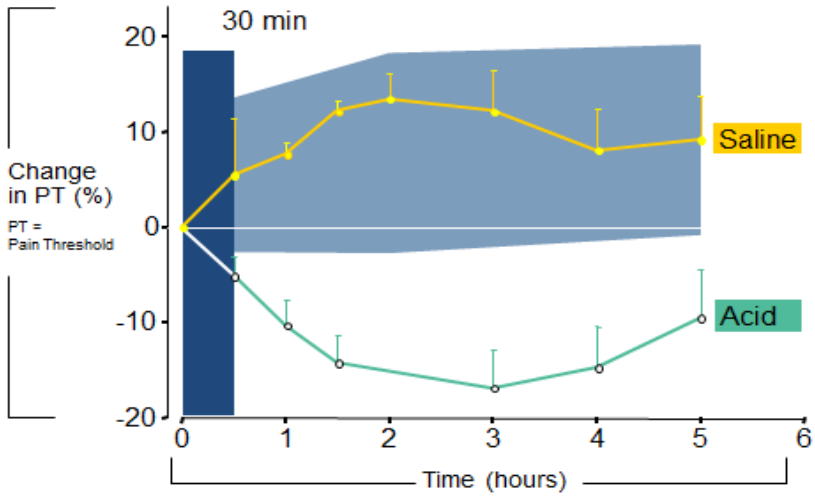


Figure 26 Mean change in pain threshold in upper oesophagus after 30 min infusion of acid or saline into the lower oesophagus in healthy volunteers, administered 2 h apart. Error bars=SE. Shaded area=95% CI calculated from change in pain threshold in upper oesophagus when no infusion was administered.
(Figure adapted from Sarkar, Aziz, Woolf, Hobson, & Thompson, 2000)(175)

Pharmacological studies have been used to block receptors involved in CS in this oesophageal model. It has been demonstrated that administration of prostaglandin receptor antagonist (EP1) prior to acid infusion blocks the subsequent development of oesophageal hypersensitivity suggesting that prostaglandins play an important role in mediating PS and CS. (176) Furthermore it was recently demonstrated that ketamine, an NMDA receptor antagonist, not only prevents development of oesophageal hypersensitivity in response to acid infusion but that it also reverses established hypersensitivity in healthy volunteers. (51) In contrast cox2 inhibitors and Neurokinin 1 receptor antagonists did not reverse the hypersensitivity in the model.

1.15 Variability in the development of oesophageal sensitisation

Despite the fact that it has repeatedly been shown that acid infusion causes oesophageal pain hypersensitivity, (175) around 15-35% of subjects do not sensitise to acid at all. (177) (Figure 27)

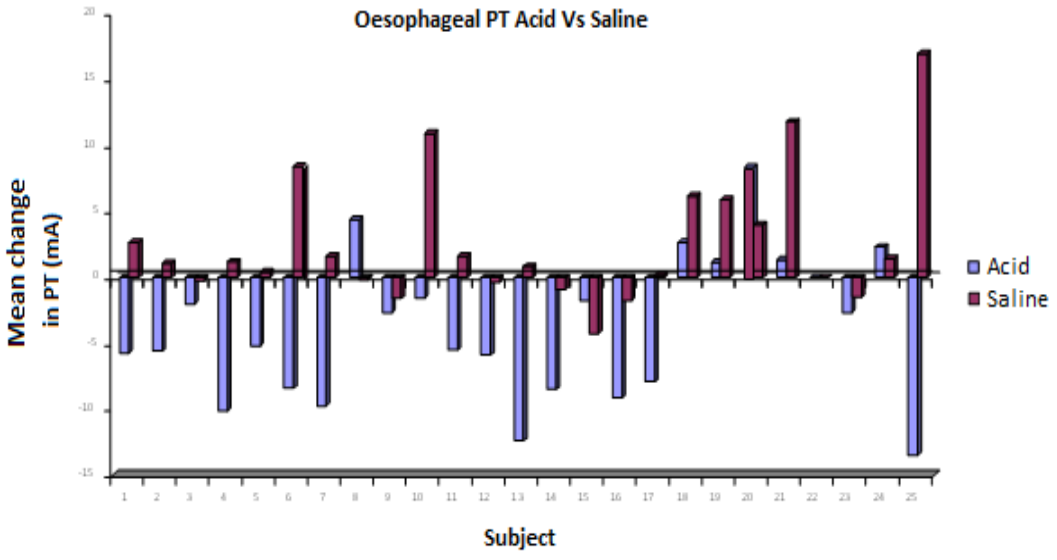


Figure 27 ***Shows the inter-individual differences in change in pain threshold (PT) after saline and acid infusions. The factors that mediate post-injury gut sensitisation are poorly understood.***

(Adappted from Sarkar, Aziz, Woolf, Hobson, & Thompson, 2000) **(175)**

Among those who do sensitise, the mean reduction in pain threshold from baseline for n=24, is -7.4mA (-8.4 to 16.3 CI, p<0.001) (177) at 30 minutes post acid infusion. This is reproducible in those sensitisers who have had more than one study. Pain thresholds remain reduced up to 120 minutes after the infusion, and there are no statistically significant differences between the pain thresholds at different time points between 30-120 minutes. The factors that mediate post-injury visceral sensitisation are poorly understood, however recent studies have shown that in healthy subjects there is variability in oesophageal pain thresholds depending on their level of state anxiety. Higher levels of anxiety are associated with lower oesophageal pain thresholds. (178) It is likely therefore that a number of psychological and physiological factors are responsible for inter-individual differences in pain hypersensitivity in this model.

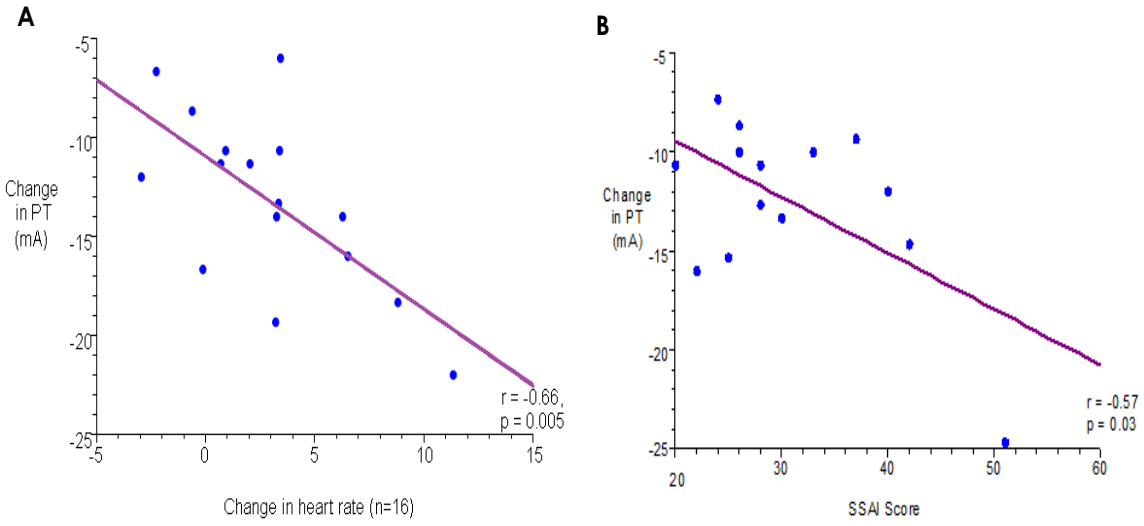


Figure 28 ***A: showing that there is an inverse correlation (r=-0.66) between the change in an individual's pain threshold (PT) and the change in heart rate, i.e. the bigger the change in heart rate, the more the drop in pain threshold. B: Correlation between baseline Spielberger State Anxiety Inventory (SSAI) score and degree of sensitisation [maximum change in proximal oesophageal pain threshold (PT) post-infusion (n=14)]. A statistically significant relationship was apparent such that as the SSAI score increased, the degree of sensitisation (fall in proximal oesophageal PT) to acid increased. (Adapted from Sharma, 2008)*** **(179)**

A study (180) of the selective activation of these systems in the human model of acid-induced oesophageal sensitisation (as described above) in 25 healthy volunteers, using novel real-time techniques for measuring the parasympathetic and sympathetic tone, has indicated the ANS may have a modulatory role on visceral pain transmission with the SNS and PNS proposed as being facilitatory and inhibitory respectively. (153, 181) Oesophageal acidification was also associated with an increase in unpleasantness and anxiety scores in conjunction with a rise in sympathetic and a fall in parasympathetic activity. Nine subjects did not sensitise to acid at all. (Figure 28(A), above) Amongst those who sensitised, subjects who showed a greater increase in heart rate during acid infusion also sensitised more. (Figure 28(B), above) Individuals who withdrew vagal (parasympathetic) tone during acid infusion the most also developed the greatest oesophageal sensitisation and resultant pain hypersensitivity. (Figure 29, below) In addition, higher state anxiety scores at baseline were associated with a greater likelihood of withdrawing vagal tone to oesophageal acidification, suggesting that these individuals may have been predisposed to greater sensitisation by their psychological state.

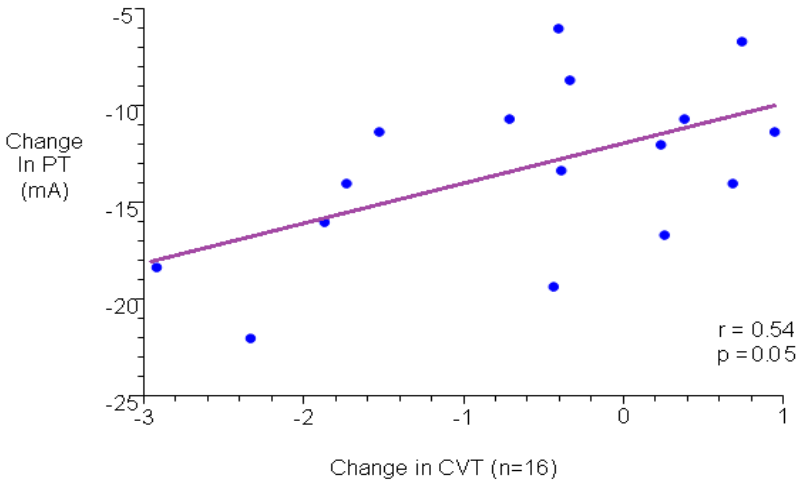


Figure 29 ***Showing the correlation between pain threshold (PT) and cardio vagal tone (CVT) measured on a linear vagal scale (LVS). (Adapted from Sharma, 2008)*** **(179)**

This data suggests that the parasympathetic nervous system may have anti-hyperalgesic properties in the human viscera, and that anxiety may predispose to greater post-injury gastric sensitisation through the withdrawal of vagal tone. The rationale then for this thesis is to take this concept one step further:

"If visceral pain hypersensitivity correlates with a decrease in cardio vagal tone; then what would the effect of a deliberate increase in cardio vagal tone be on visceral hypersensitivity?"

The plan of investigation described below is therefore based on the concept that physiological and pharmacological modulation of the ANS will lead to modulation of the oesophageal pain hypersensitivity.

1.16 Research Questions/Objectives

1.16.1 Principal research questions/objectives (Hypotheses testing)

In a model of human oesophageal pain hypersensitivity in healthy subjects:

I. Does physiological modulation of the ANS influence the degree of oesophageal pain hypersensitivity?

II. Does the increase of PNS by means of physiological methods decrease oesophageal pain hypersensitivity?

III. Does the increase of SNS by stress induction cause an increase in oesophageal pain hypersensitivity?

IV. Does inhibition of PNS by atropine cause an increase in oesophageal pain hypersensitivity due to the unopposed effect of the SNS?

1.16.2 Secondary research questions/objectives (Hypotheses generating)

In a model of acid induced human oesophageal pain hypersensitivity in healthy subjects:

I. What are the psychological predisposing factors that would predict vulnerability to VPH?

II. What is the difference in ANS response of subjects vulnerable to acid exposure compared to those that are not?

III. What is the role of psychosocial, environmental and genetic factors in ANS activation in context of VPH aetiology?

1.17 Aims

1.17.1 Hypothesis Testing

1.17.1.1 General hypothesis

The autonomic nervous system modulates the development of human VPH.

1.17.1.2 Specific hypotheses

Physiological modulation that will increase the parasympathetic tone of the ANS will decrease the degree of central sensitisation in the human healthy volunteer model of acid induced oesophageal VPH.

1.17.2 Hypothesis Generating

1.17.2.1 General hypothesis

Individual psychophysiological factors will correlate with autonomic nervous system activation that will affect the degree of central sensitisation in the human healthy volunteer model of acid induced oesophageal VPH.

1.17.2.2 Specific hypotheses

Individual psychophysiological factors that will correlate with the sympathetic & parasympathetic activation of the ANS across differing stress environments will affect the degree of central sensitisation in the human healthy volunteer model of acid induced oesophageal VPH.

2 Methods and Materials

2.1 Ethics Committee Approval and Funding

All protocols within this thesis were submitted and approved by the University Senate Ethics Committee, 'East London and The City Research Ethics Committee - Alpha' (ref: 09/H0704/71) and, where appropriate, protocols, were also approved by the Research Ethics Committee of North Jutland, Denmark (ref: N-20120065vII). Written informed consent was obtained from all subjects prior to their entry into the studies. 'Data & Identity Protection Protocols' were strictly maintained. All studies adhered to the guidelines of the World Medical Association Declaration of Helsinki (revised edition: Seoul, South Korea, 2008), the guidelines of the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (CPMP/ICH/135/95), and the 'Amendment Regulations of 2006' concerning 'Clinical Trials in Humans'. This project was funded by a Medical Research Council project grant (ref: G0701706).

2.2 Subjects

Healthy asymptomatic adult male and female volunteers, aged 18 to 50, were recruited by advertisement. All subjects were naïve to the experimental protocol and had never previously been subjected to the model of acid perfusion used in my studies. All had normal medical assessments including detailed medical interview and examination, and were non-smokers, not taking any regular medication (excluding acceptable forms of contraception). According to the "best practice" guidelines for pain research with respect to sex and gender, females were all studied in the follicular phase of their menstrual cycle. (182) Urine

tests were performed at all visits to exclude drugs of abuse (Triage 8™, Biosite San Diego USA) and pregnancy for females (First Step™ FS208 Euromed Limited, UK). Written informed consent was obtained from all after the study had been explained and only after the volunteers had in excess of 48 hours to consider the information provided. All volunteers were allowed to withdraw at any time should they wish to for any reason, or if the investigator judged that it was necessary due to any medical reasons or if non-compliance to the protocol occurred.

2.3 Oesophageal Manometry

Standardised oesophageal manometry (183) was performed in the first five subjects to determine the positions of the upper and lower oesophageal sphincter (UOS and LOS) from the nostril. A stationary pull through manometric technique was performed by a research assistant accredited in the procedure. Intraluminal pressures were measured via 4 channels (0.9mm diameter) incorporated into the solid state catheter, the ends of which opened as side holes 5, 10, 15 and 20cm from the distal tip of the catheter (Polygram™ for Windows® 1995, Synectics Medical, Enfield, Middlesex, EN1 3BT, UK). This measurement was then compared with the measurement obtained using a stationary pull through 'pH change technique'. A 1mm diameter twin channel pH catheter (Synectics Medical, Enfield, Middlesex, EN1 3BT, UK) was used to measure the relative LOS position indicated by the pH change as the pH catheter was slowly withdrawn from the stomach. The LOS positions on these first five subjects were found to be identified accurately enough by the pH change technique for the purpose of this study, and only the 'pH change' pull through technique was used for the remainder of the subjects. (Specific corrections in possible case of hiatus hernia were not made.) Determination of LOS position was essential for later positioning of the stimulation and infusion catheter assembly.

2.4 Catheter Assembly

All experiments were conducted with subjects having fasted for a minimum of 8 hours. A bespoke naso-oesophageal catheter consisting of two electrical stimulation electrodes 15cm apart with a infusion port 1cm above the lower electrical stimulation electrode, (UniTip™-Katheter 6F, UNISENSOR AG, Attikon, Switzerland) were taped together with a disposable 15cm twin channel pH catheter (VersaFlex® Sierra Scientific Instruments, LA, CA, USA). The catheter-pH probe assembly was passed nasally into the oesophagus until the distal infusion site and the proximal stimulating electrodes were 4cm and 18cm above the lower oesophageal sphincter respectively, with the pH sensors sited adjacent to the infusion and stimulation sites (Figure 30; and Figure 38(f)). Local anaesthetic spray was not used to avoid contamination of the proximal oesophagus, which in turn may have affected the sensory measurements, but passage was eased through the naso-pharynx with a water-based lubricant jelly (KY jelly™, Johnson & Johnson).

To ensure correct placement, a 'Flush through-test' was performed, whereby 10ml sterile water was injected via the infusion port into the oesophagus. The subject was then closely observed to ensure that the cough reflex was not triggered, and was asked to respond to a few questions to ensure that they could speak with unobstructed vocal chords. The reusable infusion-simulation catheter was sterilised (Pera safe™, Antec International- a DuPont company, Suffolk, UK) at the end of every experimental session.

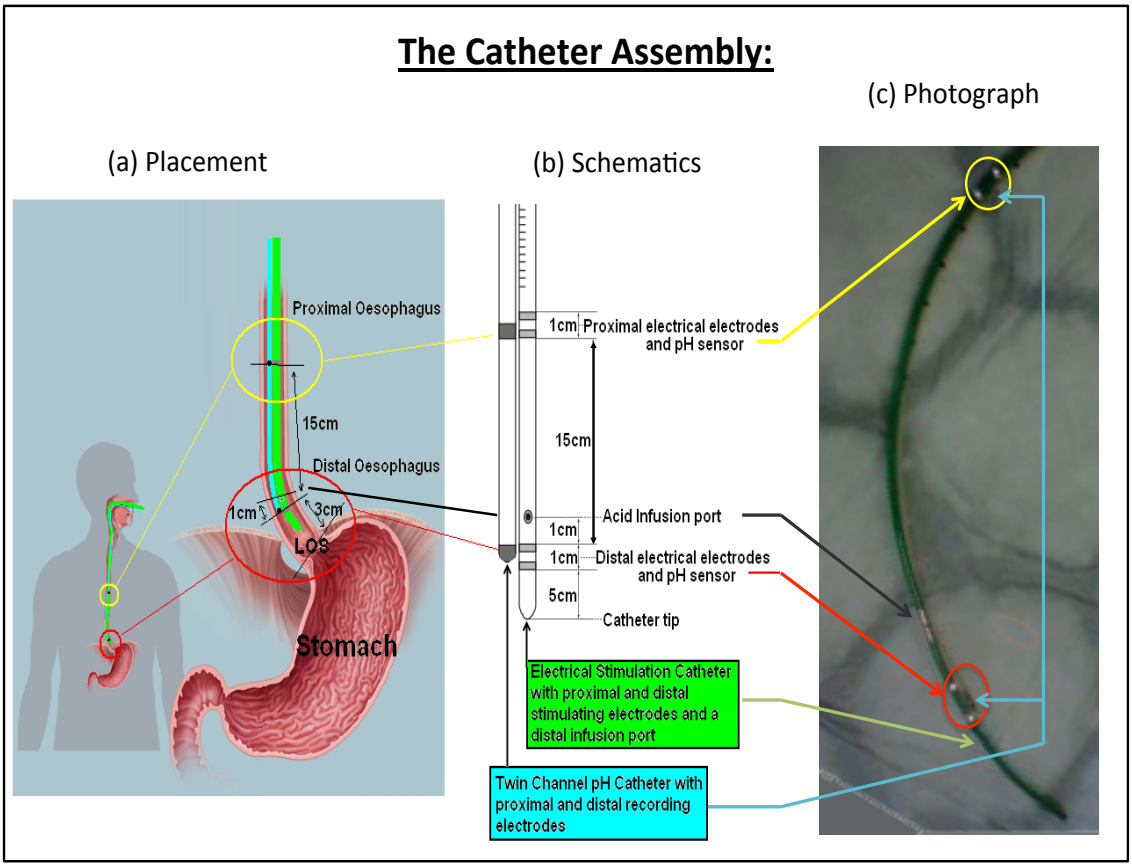


***Figure 30** The naso-oesophageal catheter assembly, consisting of a pH probe (blue label) and an infusion-stimulation catheter (green label) strapped together. Illustrated is (a) the positioning in the oesophagus, (b) its schematic proportions, and (c) a photograph of the actual assembly.*

2.5 Oesophageal acid infusion

Four 60ml disposable syringes were pre-loaded with 0.15M hydrochloric acid (HCl) (Stockport Pharmaceuticals, Stockport, UK) which was warmed to body temperature in a water bucket priority to the infusion and then infused via an infusion pump (Omni fuse™, Graseby Smiths Medical Inc. MN, USA; see Figure 38(i)) into the distal oesophagus, 4cm above the LOS, through the infusion port of the infusion-stimulation catheter at a constant rate of 8ml/min for 30 minutes (Figure 31) to a total infusion volume of 240mls. The proximal oesophagus remained acid-free (pH >4) while the distal oesophagus was exposed to acid (pH < 2).

2.6 Oesophageal pH monitoring

A 1mm diameter twin channel pH catheter (VersaFlex® Sierra Scientific Instruments, LA, CA, USA; see Figure 30 and Figure 38(f)) continuously measured pH in the proximal and distal oesophagus (at the sites of acid infusion and electrical stimulation respectively) for the duration of each study. Recordings were made using a twin channel pH box (Synectics Medical™, Enfield, Middlesex, EN1 3BT, UK; see Figure 38(l)).

2.7 Visceral Pain Hypersensitivity Model

Sarkar *et al.* (175) have developed a robust healthy volunteer model of human oesophageal sensitisation as illustrated by Figure 31. In this model, to explore mechanisms of visceral hypersensitivity in the oesophagus, acid is infused in the distal oesophagus. Subsequent pain hypersensitivity to electrical stimulation has been demonstrated in the distal acid-exposed region (primary hyperalgesia), the proximal non-acid-exposed oesophagus and the area of somatic referral on the anterior chest wall (secondary hyperalgesia). (175) Oesophageal pain hypersensitivity has been repeatedly shown to occur following acid infusion using this model in several studies. (184-190) A significant variability in developing this sensitisation has been recorded, with around 15-35% of healthy volunteers not being sensitive to acid infusion. (51) This model provides a validated reproducible basis for standardised comparative study into the underlining mechanisms and modulators of acid sensitisation; and as such, was ideally suited for use in this study.

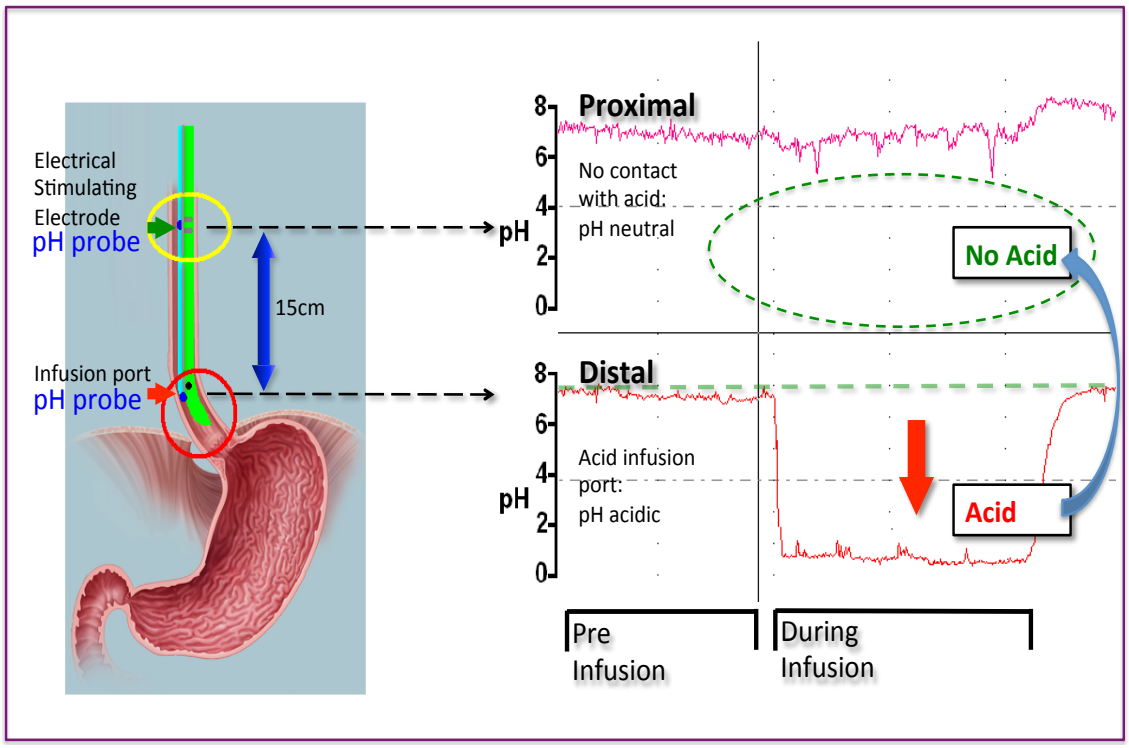


Figure 31 ***The naso-oesophageal catheter assembly, illustrating with actual pH-metry that the distal pH drops during acid infusion while simultaneously maintaining an acid free environment 15cm proximal in the oesophagus in a fasting volunteer, where the pain tolerance threshold changes are measured.***

2.8 Sensory and Pain Threshold Measurements

Sensory and pain thresholds to electrical stimulation were determined in the proximal oesophagus (18cm above the lower oesophageal sphincter), the distal oesophagus (3cm above the lower oesophageal sphincter), and foot (somatic control). Oesophageal sensory testing was performed via a pair of silver-silver chloride bipolar ring electrodes (inter-electrode distance 1cm) sited proximal to the tip of a 3mm diameter catheter (UniTip™-Katheter 6F, UNISENSOR AG, Attikon, Switzerland). Stimulation consisted of electrical impulses of increasing strength delivered using a constant current stimulator (Digitimer™, model DS7A, Digitimer Ltd, Hertfordshire, England; see figure 30(j)). An established stimulation protocol was used based on previous studies. (53, 175, 191) The intensity of the stimulus was increased in a step-wise manner by 2mA

(at 200V) intervals, beginning at an intensity of 0mA up to a maximum of 98mA. Stimuli were delivered at a frequency of 0.5Hz (i.e. 1pulse/2second), using square wave pulses 500μs[8] in duration. At each site, three measurements of sensory and pain tolerance were recorded, 60 seconds apart, and the mean value calculated (Figure 30).

For each stimulus set, the sensory threshold at which the subject felt the sensation, as well as the pain tolerance, was recorded. Subjects were instructed that this should be the level beyond first pain sensation at which they could not tolerate further increase. Hence this is most accurately described as a pain "intolerance" threshold, i.e. that at which they become intolerant of pain, rather than a pain "tolerance" threshold, i.e. the last level at which they can still tolerate it. This level was equivalent to a rating of 7 on a Visual Analogue Scale (VAS) (192) (Figure 32) ranging from 0 (no sensation) to 10 (unbearable pain). This scale shows a linear relationship with that of the pain descriptor and stimulus intensity. (193)

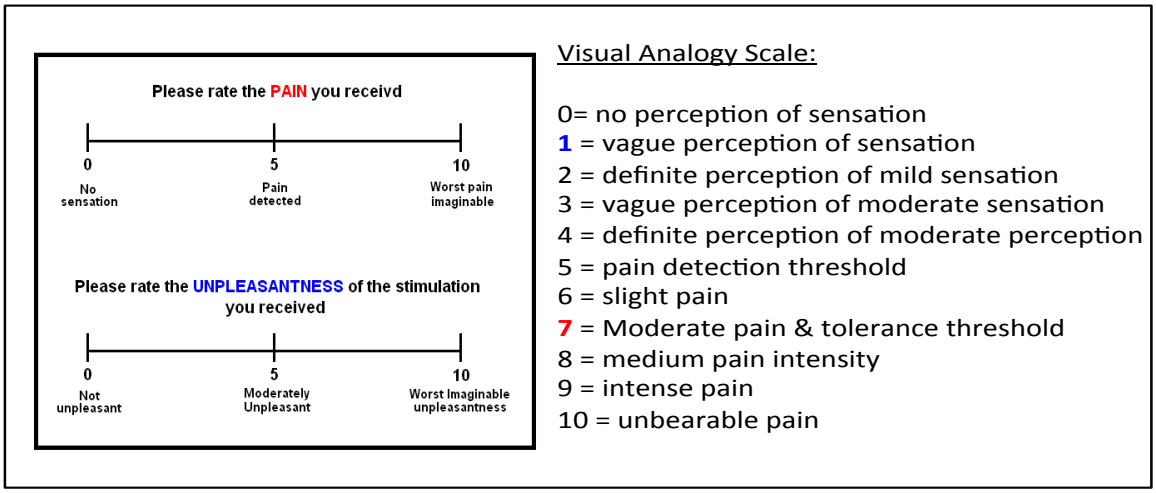


Figure 32 ***The 11-point VAS for the quantification and measurement of the pain and unpleasantness. This was used after every sensory and pain threshold measurement. (Adapted from Drewes, 2003)*** **(192)**

[8] For a more detailed description see appendix one (2).

Electrical stimulation was immediately stopped when pain intolerance threshold was indicated. Control (somatic) pain thresholds were taken in an identical manner using a pair of disposable surface electrodes (Oxford Instruments, Medical Systems Division, Woking, Surrey, GU22 9JU, England; see Figure 38(e)) that were placed on the dorsum of the right foot 2cms above the 4th metatarsophalangeal joint.

2.9 Pain Tolerance Threshold Calculation

Using measurements taken in the proximal oesophagus at each time point, baseline, prior to acid infusion (T0), then 60 minutes (T60), 90 minutes (T90) and 120 minutes (T120) post acid infusion, pain tolerance threshold (PT) was used to characterise subjects as either sensitisers or non-sensitisers. The change in PT (Δ) was determined by calculating the mean of the three pain tolerance threshold measures at each post infusion time point (T60, T90 & T120) and then subtracting this value from the mean of the three pain threshold values, prior to acid infusion (T0). (185, 194) (Figure 33)

The three values thus obtained were then averaged to obtain the PT. Subjects were classified as sensitisers if there was a fall in the proximal oesophageal pain threshold (PT) of ≥ 6mA after distal oesophageal acidification during a non- modulation visit (i.e. screening- or sham breathing protocols only; see section 2.20), as compared with the pre-infusion threshold. They were classified as non-sensitisers if the decrease of proximal oesophageal PT was < 6mA during a non-modulation visit. (185, 194)

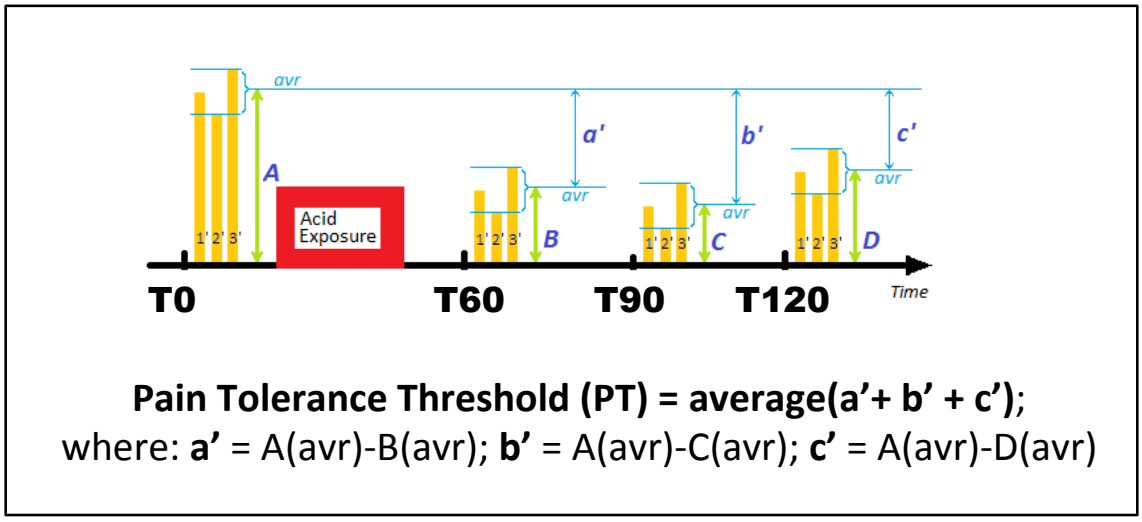


Figure 33 *This diagram shows the calculation of the ∆Pain Tolerance Threshold (PT). A represents the mean of the three-baseline pain threshold measures at T0. B, C & D represent the average at T60, T90 and T120. The difference between A-B is represented by a' and A-C as b' & A-D as c'. The PT therefore represents the mean of the values for a'+b'+c'.*

2.10 Cannula insertion and blood sample

An 18 gauge (green) intravenous cannula was inserted into the right antecubital fossa prior to the nasogastric intubation, where appropriate (Figure 38(d)). This was used to administer the atropine or placebo, 5 minutes prior to starting the acid infusion. The cannula was also used at the end of the study when a 5ml blood sample was obtained and frozen at -80°C. This sample was later used to prepare an assay for genomic DNA extraction and genotyping.

2.11 Psychological assessment

During the first visit, subjects completed a set of computer-administered profiling, state and trait questionnaires. (Figure 38(k)) For all subsequent

visits, only a state questionnaire was completed. The questionnaires included the:[9]

2.11.1 Big Five Inventory (BFI)

The 44-item questionnaire (195) allows efficient and flexible assessment of the 5 dimensions of personality, i.e. extraversion, agreeableness, conscientiousness, neuroticism and openness to experience. Essentially, the big five inventory is used to explore the broad factors of personality traits developed through factor analysis of a large population through empirical research. As previously demonstrated in preliminary studies, the personality of traits of extraversion and neuroticism have an association with autonomic nerves system (ANS) responses to visceral pain. (196, 197)

2.11.2 The Weinberger Adjustment Inventory (WAI)

This is a well-validated trait measure of repression of negative affect. It measures the way in which an individual reacts to conflict and stressful situations through three dimensions; distress (anxiety, depression, low self-esteem, low well-being), restraint (suppression of aggression, impulse control, consideration of others and responsibility), and defensiveness (repressiveness, denial of distress). (198)

2.11.3 Toronto Alexithymia Scale (TAS- 20)

The TAS is a 20-item instrument that is one of the most commonly used measure of alexithymia. Alexithymia refers to people who have trouble identifying and describing emotions and who tend to minimise emotional experience and focus attention externally. Research using the TAS-20

[9] For the questioners see appendix two.

demonstrates adequate levels of convergent and concurrent validity. (199)

2.11.4 Hospital Anxiety and Depression Scale (HADS)

The 14-item questionnaire was designed as a screening tool to detect depression and anxiety. It consists of 14 questions with seven related to anxiety and seven related to depression. It was originally designed for use in general hospital outpatients but has been extensively used and validated in primary care. (200)

2.11.5 Spielberger State (SSAI) and Trait (STAI) anxiety Questionnaire

The Spielberger state and trait anxiety questionnaire is a widely used self-report questionnaire. As one may expect, the state questionnaire asks how the subject feels at the present moment, whereas the trait questionnaire enquires about long-term feelings of anxiety. Some authorities suggest that trait anxiety and neuroticism are mutually exclusive. (201)

2.11.6 Vulnerable Attachment Style Questionnaire (VASQ)

The Vulnerable Attachment Style Questionnaire was developed to provide a brief self-report research tool to assess adult attachment style in relation to depression and validated against an existing investigator-based interview (Attachment Style Interview – ASI). (202) It is based on Attachment theory (119), which describes the dynamics of long-term relationships between humans. It explains how the parents' relationship with the child influences development and becomes the basis for later attachment behaviour known as the adult attachment style.

2.12 Measurement of the Autonomic Nervous System

The role of the ANS in the pathophysiology of a number of disorders including cardiovascular mortality and chronic pain has only been recognised and fully appreciated in the last three decades. (203) Partly the problem has to do with the lack of suitably sophisticated technology to measure and study the ANS and its systemic impact. Using an inserted needle recording of the peroneal nerve the ANS function can be measured directly. Similarly the vagus nerve can be facilitated through subcutaneous pacemakers-like stimulators. These methods are unfortunately very invasive and impractical for experimental studies. The need has arisen to develop accurate indirect measures by which ANS function can be monitored. The most popular method has been the heart rate variability (HRV). In the following section is a critique of the theory underlying HRV, and a description of more novel non-invasive, beat-to-beat measures of autonomic tone used in this thesis.

2.12.1 Heart Rate Variability (HRV)

Early studies related HRV to physiological mechanisms, and only a few historical studies highlighted the emergence of HRV as a physiologically meaningful measure. An example of this is Wundt (204), who used HRV to study repertory sinus arrhythmia (RSA). As interest in HRV increased, it was used both as an individual difference variable in obstetrics, paediatrics, developmental psychology, psychiatry, and health psychology and as a response variable in ergonomics, human factors engineering, and cognitive sciences.

Almost all the studies investigating HRV have occurred during the past 40 years. Clinical interpretations and applications have an even shorter

history. The linking and integration of central nervous system structures to autonomic functions, as seen in theories like the Polyvagal Theory (100, 108), have only emerged during the past few decades. (91, 93, 98, 205-207) Presently a non-physiological "operational" model still dominates the literature and still influences how HRV is quantified and interpreted in the literature. For example, various strategies to quantify RSA have focused on phenomenological features (e.g. relation to respiration) and not on neurophysiological (e.g. medullary interneurons, neuropeptides, neurotransmitters) or neuroanatomical features (e.g. source nuclei of vagal efferent pathways). In 1965 Hon *at al.* (208) demonstrated that foetal distress was predicted by alterations in the inter-beat intervals between successive R waves in the electrocardiograph (ECG), before detecting any changes in the heart rate (HR). This highlighted the direct clinical relevance of HRV for the first time, and since then the majority of research in autonomic nervous system has preferred using the HRV rather than the crude HR. HRV analysis has mostly been done by means of two methods; the time domain analysis and the frequency domain analysis with its emphasis on the power spectrum density (PSD).

2.12.2 Time Domain Analysis

In a continuous ECG recording, the interval between consecutive normal QRS complexes on the ECG is known as the normal-to-normal (NN) interval. Two statistical classes are derived from the normal-to-normal interval. The first class uses direct measurement of the NN intervals, while the second focuses on the differences between the NN intervals. The simplest variable is the SDNN (standard deviation of normal-to-normal RR intervals). (209) This value reflects the variability of the cyclic components in an ECG recording. Other commonly used measures are detailed in Table 1. This method's major disadvantage is its statistical power

limitations, as it only allows for short-term recordings (less than five minutes) to be evaluated. (118, 210)

Table 1 Commonly used domain analysis variables. *(From Farmer, 2010)* (211)

Variable (units)	Description	Physiological Relevance
SDNN (ms)	Standard deviation of the normal RR (NN) interval reflecting all of the cyclic components responsible for variability in the period of recording.	An overall estimate of HRV, but does not indicate the contribution of any particular influence.
SDANN (ms)	Standard deviation of the averages of NN intervals calculated over a short period of time, usually less than five minutes.	Reflects the influence of circadian rhythms on autonomic function.
pNN50 (%)	The proportion of NN intervals having a difference of >50mSec.	Reflects predominant vagal influence on variability.
Triangular Index (ms)	The integration of the density distribution of all the NN intervals as a function of the maximum density.	Overall estimate of HRV similar to SDNN.

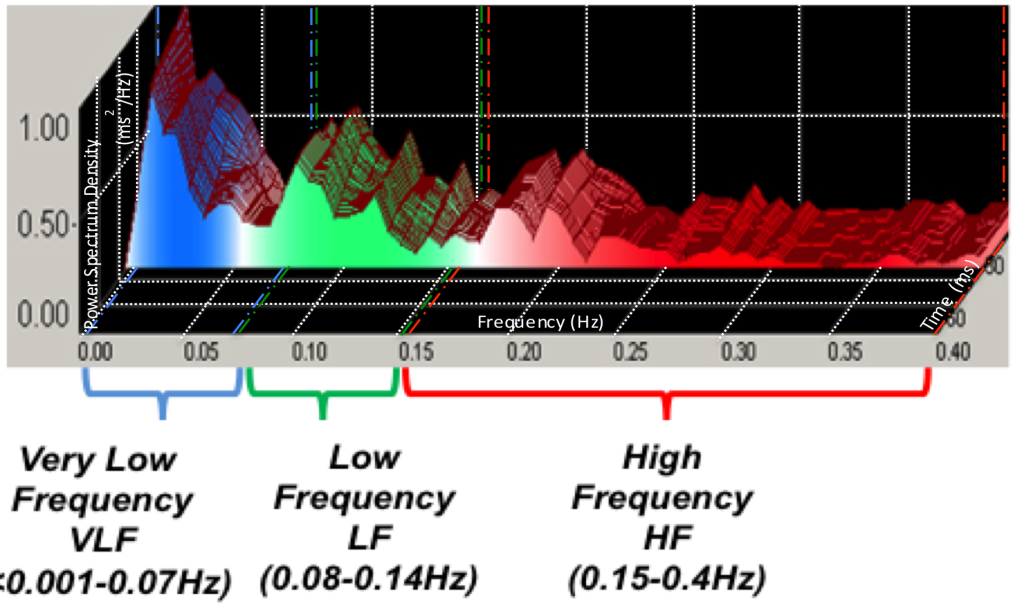
2.12.3 Spectral Domain Analysis

The sympathetic and parasympathetic components of the autonomic nervous system oscillate at different frequencies, and hence can be distinguished by means of the quantitative breakdown (power) of the different frequencies influencing the HRV. This is known as the power spectral density (PSD) and has become one of the preferred methods of analysing the autonomic nervous system to date. (212) When considering short-term recordings claimed in resting conditions (3 to 5 minutes) the PSD is subdivided into three main frequency bands: high-frequency (HF: 0.15-0.4Hz), low-frequency (LF: 0.08-0.14Hz) and very low frequency (VLF: 0.003-0.07Hz). (Figure 34) Some researchers also distinguish a fourth band known as the ultralow frequency (ULF: <0.003Hz). These rhythms can be divided further and are considered to reflect as demonstrated in Figure 34, below. (210, 212, 213)

The term cardiac vagal control (CVC) refers to the vagal output to the heart and it is thought to correlate with respiratory sinus arrhythmia (RSA) that is represented by the HF band. A further interesting phenomenon of this is that when the respiratory rate is between 10 to 12 breaths per minute (0.16-0.2Hz), the RSA assumes semi-stationarity. Due to this phenomenon, experimenters need the co- operation of the subject, in controlling their breathing rate when recording for normal physiological analyses. When the respiratory rate reaches about 6 breaths per minute by means of 'paced breathing' techniques, it can be observed within the HF band (±0.1Hz) and is referred to by some authors as achieving 'resonance', and maximises the CVC. (213) A central methodological criticism of this measure of CVC was highlighted by Denver, who commented that, "*…techniques such as paced breathing artificially elevate the CVC making its measurements unreliable…*" and is hence ambiguous with regard to normal physiological analyses. (208) Of note is that this also creates the ideal opportunity to stimulate the CVC and increase parasympathetic tone when breathing at a similar frequency of 0.1Hz (i.e. 6 breaths per minute). (213) (See section 2.20.3, page 114)

A further complicating factor in using the PSD is that the physiological mechanisms responsible for the modulation of LF and HF components cannot be considered to be stationary for long HRV recordings, in particular those over 24 hours, due to the aforementioned difficulties associated with 'stationarity-assumptions'. Problems are not confined to respiratory frequency only, for example in the literature, spectral analyses recordings taken over such periods of time are often reported in a single time block, i.e. the whole 24-hour period, or in shorter segments, usually five minutes, with the results averaged over the whole time period. (212) Hence, spectral analyses performed in either of these periods provide averages of the modulations attributable to the LF and HF components,

but such averages obscure detail regarding specific autonomic modulation.



ULF:	*- Circadian rhythms (not separately illustrated above).*
VLF:	*- Thermoregulatory cycles, with a sympathetic dominance.*
LF:	*- Mixed sympathetic and parasympathetic control, although there is a parasympathetic dominance. (Paced RSA control at 0.1Hz)*
HF:	*- Respiratory frequency in the resting healthy adult human, which is under parasympathetic influence. (Spontaneous RSA control at >0.15Hz)*

Figure 34 Power Spectrum Density (PSD) frequency bands, with physiological correlations. ***Below is illustrated a 3D-PSD (change over time, z-axis) of a healthy young subject during supine rest.*** **(213)** ***(Adapted from McCarty, 2009) (210, 213)***

Finally, spectral analysis provides a representation of modulatory influences rather than autonomic tone as such (see beat-to-beat measure in section 2.12.5), as it provides a degree of the autonomic modulation of HRV by its different components, rather than the level of autonomic tone *per se*.

2.12.4 LF:HF – The Sympathovagal Balance Controversy

Many studies in the literature derive a measure of sympathovagal balance, through the examination of the ratio between LF and HF. This method is potentially unclear, due to the assumption of two factors: (i) that LF and HF purely represent the sympathetic and parasympathetic modulation of HRV, and (ii) that there is direct reciprocity between LF and HF. (203)

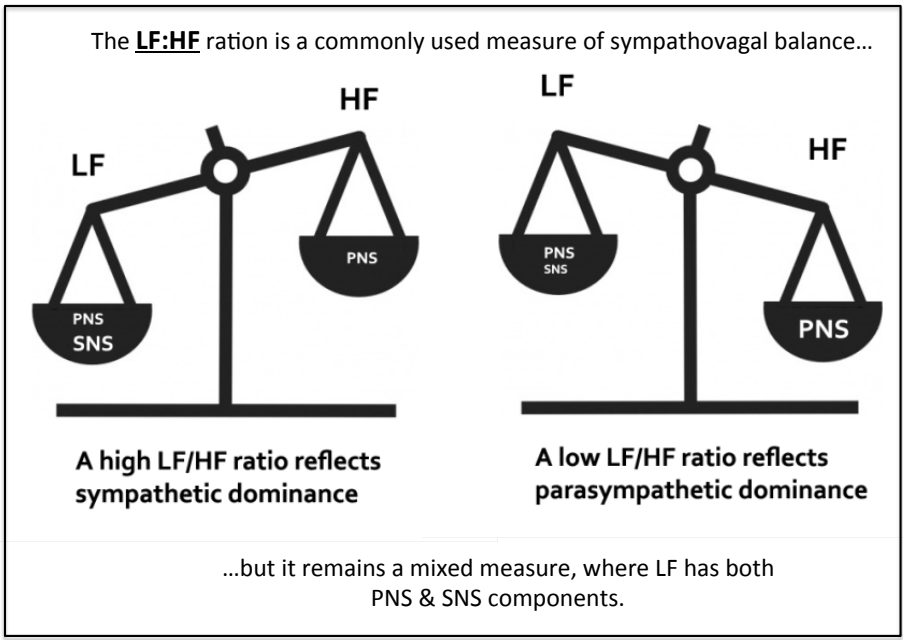


Figure 35 ***This diagram illustrates the methodological short-comings of using the LF:HF ratio as sole measure of sympathovagal balance in HRF analysis.***
(Adapted from McCarty, 2009) (213)

For a number of reasons these assumptions are not totally scientifically sound. Eckberg and colleagues demonstrated that blocking the vagal component of the LF with atropine had little effect on LF, whereas the converse was evident with sympathetic blockade. (214) There is thus a greater parasympathetic influence in the LF in comparison to the sympathetic nervous system. (Figure 35) Based on similar findings, Porges *et al.* suggested that HF might reflect CVC from the nucleus ambiguous

(NA), whereas the LF may reflect CVC from the dorsal motor nucleus (DMNX). (215) Porges designed a moving polynomial filter in an attempt to redress these methodological issues. (216) It entails a complex statistical method that utilises a time domain approach (moving averages) and smoothing filters to evaluate dynamically the rhythmic oscillations of the varying RR frequencies.

In spite of Porges' technique having the added value of pre-existing sex and age normal values for humans, the filter is not easily practically applied. This technique's temporal resolution is poor beyond one minute for LF and two minutes for HF. To be exact, it has been recommended that this method of analysis is not used for more than two minutes' worth of data. Despite these drawbacks, the LF:HF ratio of the sympathovagal balance remains a commonly utilised method of measurement in autonomic neuroscience research.

2.12.5 Overcoming ANS Measurement Difficulties

In order to overcome the aforementioned difficulties beat-to-beat measures were developed, as they represent direct measures of autonomic tone, irrespective of time frame or assumptions of respiratory stationarity. Examples of beat-to-beat measures are cardiac vagal tone (CVT) and cardiac sensitivity to the baroreflex (CSB).

2.12.6 Measuring Cardiac Vagal Tone (CVT)

The measure of parasympathetic stimulation to the heart via the vagus nerve is known as CVT. The momentarily blood pressure (BP) increase during ventricular systole causes baroreceptor activation in the carotid sinus and pulmonary circulation to increase their rate of discharge. (217)

In turn, via medullary neurones in the nucleus of the solitary tract (NTS), a vago-vagal reflex is initiated, which then stimulates vagal preganglionic neurones to increase firing. This increased cardiac vagal activity causes a reduction in the rate of spontaneous depolarisation of the sino atrial node, and widens the RR interval and decreasing HR. As the vagal response to baroreceptor stimulation in humans takes about 240ms, it is fast enough to delay the subsequent systole, resulting in beat-to-beat changes, known as heart rate variability (HRV). (218) (Figure 44) (This physiology underpins the clinically used measure of carotid sinus massage as a "vagal manoeuvre" in the treatment of supra-ventricular tachycardia.) Thus, even though the SNS influences the HR, for example through changes in peripheral vascular resistance which takes place more slowly, it is possible to deduce vagal tone in a non-invasive manner by measuring of the beat-to-beat changes in RR intervals.

Based on these principles, the NeuroScope™ (MediFit Diagnostics Ltd, London; see Figure 38(a)), is a novel piece of technology that analyses the RR interval to produce the real time index of parasympathetic activity known as CVT. (219) A standard 3 lead ECG is recorded and the Neuroscope samples this ECG waveform at 5kHz. The acquired QRS complexes are then compared to a QRS template generated from the initial stages of the recording. If there is sufficient similarity between the recorded complex and template, a 1mV pulse is generated by voltage oscillators. Thus, the time between 1mV pulses is equivalent to the RR interval on the ECG. The Neuroscope circuit sends this pattern of 1mV pulses into two circuit limbs known as the high pass limb and the low pass limb. The high pass limb tracks the incoming signal without transforming it, whereas the low pass limb produces a damped version of the signal.

The lower the rate of HRV, the slower the rate of change of the incoming signal, and the closer the output match between the high and low pass limbs, and the lower the CVT. Conversely, the higher the HRV, i.e. the faster the rate of change of the incoming signal, the more damped the low pass circuit output is in comparison to the high pass limb, resulting in a higher CVT reading (Figure 36). This process has been termed phase shift demodulation.

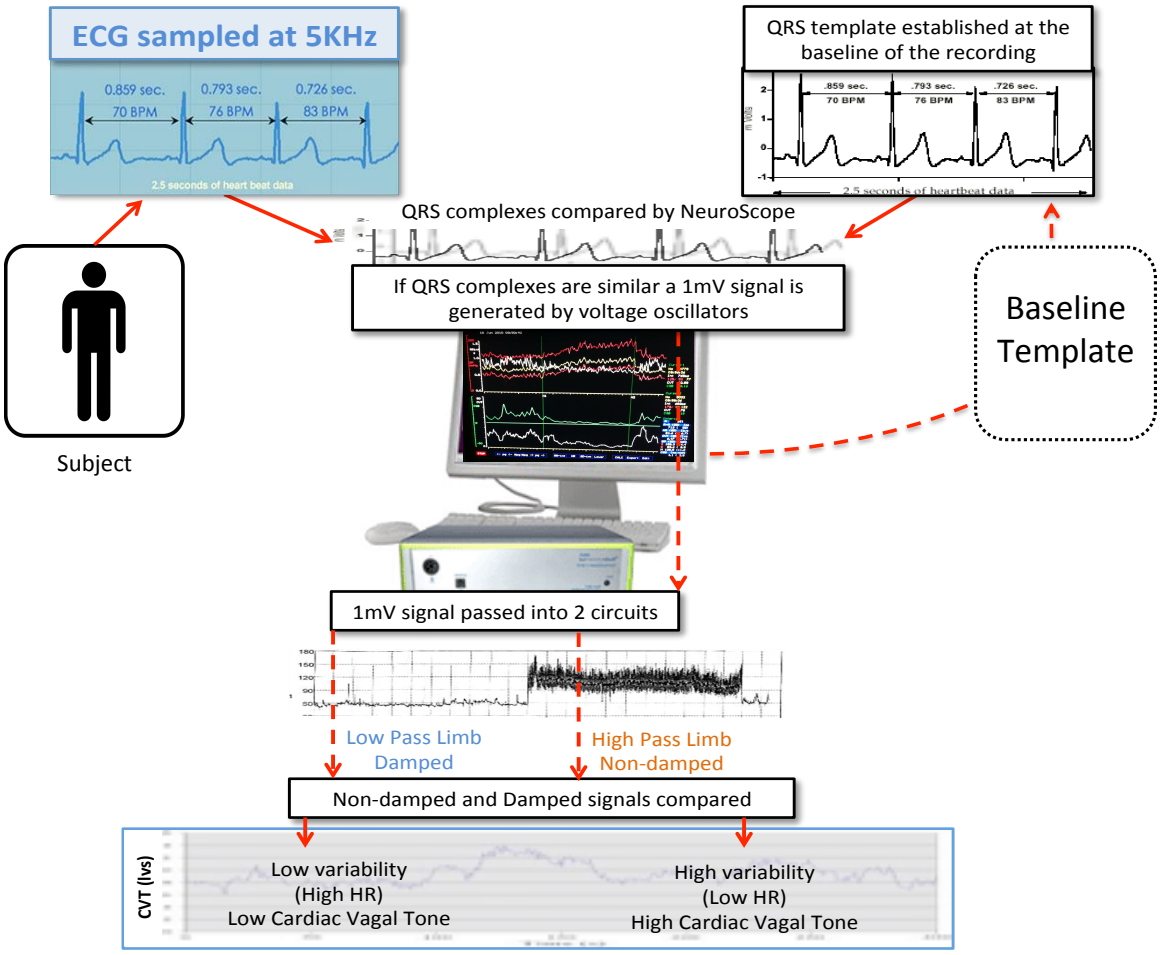


Figure 36 ***The beat-to-beat measure of cardiac vagal tone (CVT) as measured by the Neuroscope, using voltage oscillators with high (non-damped) and low (damped) circuit limbs. CVT variability is calculated on a linear scale (Lvs).***
(Adapted from Farmer, 2010) **(211)**

This methodology of measuring CVT has been validated in humans and animals. (10) CVT is measured on an experimentally derived linear vagal

scale (Lvs). Zero on the Lvs was derived from six fully atropinised healthy volunteers, and 10 units on the Lvs established in the same volunteers in the supine position in the fasting state (i.e. maximal vagal activity). (219) Thus, CVT can be considered a validated marker of efferent parasympathetic tone from the brainstem on the heart.

2.12.7 Cardiac Sensitivity to the Baroreflex

In addition to the derivation of CVT, the Neuroscope also measures CSB, a validated, non-invasive beat-to-beat measure of parasympathetic afferent activity. Incorporated in the Neuroscope system is a non-invasive continuous BP measurement using the Portapress™ system (Finapress®, Amsterdam, Netherlands). From this, the Neuroscope uses the raw Nexfin® waveform to calculate the arithmetic mean of the blood pressure (MBP), as opposed to the mean arterial blood pressure (MAP) that is commonly used in the clinical setting (MAP = DBP + 1/3(SBP – DBP); where DBP & SBP is diastolic and systolic BP respectively). The MBP that is calculated by the Neuroscope is the true arithmetic mean of the BP, i.e. DBP, dicrotic notch and the SBP. By integrating the RR interval data with the BP data, the change in pulse interval per unit change in SBP over a 10-second period can be calculated; this is termed CSB, which is expressed as a ratio of mmHg/ RR interval (ms/mmHg). (219)

From section 2.12, it is clear that the Neuroscope allows the beat-to-beat measures of both the efferent and afferent limbs of the parasympathetic tone on the heart, without the methodological difficulties that are associated with spectral analysis of HRV.

2.13 Selective Sympathetic Measures

2.13.1 Vasomotor – arithmetic Mean of the Blood Pressure (MBP)

Mean arterial pressure (MAP) has been shown to correlate with invasively recorded sympathetic activity, as assessed by photo-plesythymography. (220) Photo-plesythymography records MAP on a beat-to-beat basis and has been validated against invasive arterial pressure measurements in humans. (221) However, it must be noted that if the cuff is applied to a subject's finger for a considerable period of time, a degree of vasoconstriction can ensue. Selecting the correct size cuff is of utmost importance, as selecting the wrong size can result in large fluctuations in BP readings. The BP cuff was placed on the subject's left middle finger in this series of experiments. The analogue readings from the Nexfin® were transmitted to the Neuroscope, where they were digitised and integrated into the beat-to-beat data, as discussed. Blood pressure was measured continuously with a Finometermodel 2, Finapress® Medical Systems™, finger cuff and a Portapress™ non-invasive blood pressure monitor (Finapress™ Medical Systems, Amsterdam ZO, The Netherlands (Figure 38(g)).

2.13.2 Sudomotor – Skin Conductance Response (SCR)

The sudomotor, or skin conductance response (SCR), (222) measurement has been used for more than 100 years, and is a measure of selective central sympathetic control over sweat gland activity. It can be defined as the

> "…*momentary change of the electrical potential of the skin, [it] may be spontaneous or reflexively evoked by a variety of internal or by externally applied arousal stimuli.*" (223)

This simple electro- physiological measure assesses sympathetic cholinergic sudomotor function, and represents a transient change in the electrical resistance of the skin that is associated with sweat gland activity elicited by a stimulus that evokes an arousal or orienting response. Although human neuroanatomical efferent sweat pathways have not been fully determined yet, animal studies have shown that efferent sweat fibres originate in the hypothalamic preoptic sweat centre and descend through the ipsilateral brainstem and medulla to synapse with the intermediolateral cell column neurons. Unmyelinated postganglionic sympathetic class C fibres arise from the sympathetic ganglia to join the major peripheral nerves and reach the sweat glands. (223) There are two interacting types of sweat response, namely thermal and emotional. Emotional (mental) sweating control has multiple interactions, with emotional, cognitive and neuroendocrine functions, and is controlled at multiple levels within the CNS, mainly at the ACC.

There are two main methods of SCR acquisition, firstly to measure spontaneous impedance changes across digits (often referred to as galvanic skin responses or "GSR"), or secondly to pass a small, constant current across the digit and record impedance changes as it crosses the digit (usually called the SCR) – with the latter felt to be more reliable. (222) The Powerlab™ (AdInstruments, UK) biosignals acquisition (Figure 38(b)&(c)) system can digitally record SCR, which were recorded at baseline and during acid infusion. The SCR electrodes were placed on the subject's left index and ring finger in this series of experiments. (Figure 38(h))(224) SCR is measured in micro-Siemens (mS), and is a measure of pure sympathetic sudomotor activity. (Figure 37)

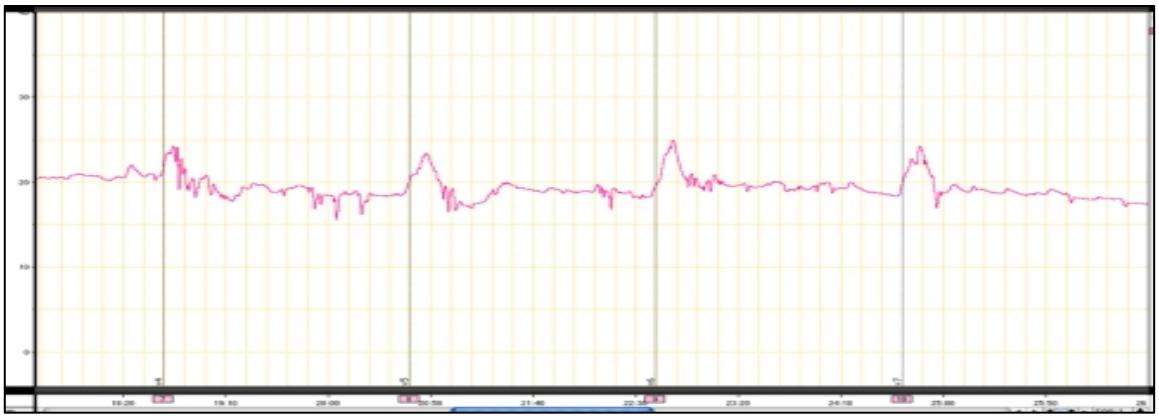


Figure 37 ***A typical SCR tracing. The black vertically dashed lines represent the application of a noxious stimulus, and the red line represents the SCR trace*****.**
(Adapted from Farmer, 2010) **(211)**

2.14 Summery of Autonomic Measures and Recordings

The Neuroscope was used to make all ANS recordings during the set of experiments described in this thesis, and CVT and CSB were taken as the main measures of parasympathetic activity in subjects. (225, 226) This facilitated the study of both temporal and causal relationships of brainstem responses to external stimuli, as mentioned above. The CVT is measured and quantified in units of a linear vagal scale (Lvs) (219), whereas CSB is defined as the increase in pulse interval per unit increase in systolic blood pressure and is expressed as R-R interval (ms/mmHg). The full complement of autonomic measures used in this series of studies covers mixed measures (HR), parasympathetic efferent tone from the brainstem (CVT), parasympathetic afferent tone (CSB), sympathetic vasomotor (MBP) and sympathetic sudomotor (SCR).

During experimental recordings, participants were instructed to remain strictly motionless and quiet while seated in a fully supported 80° upright examination couch (not illustrated in Figure 38 below) to minimise artefacts on autonomic recordings. The first five-minute pre-intervention baseline recording was acquired before intubation or cannula insertion,

and was used for inter-group comparisons. After intubation and a 10-minute rest period a second five-minute baseline recording was acquired and used to compare with a 30-minute recording performed during the infusion period. Figure 38 (below), demonstrates the equipment and their attachment layout.

Figure 38 ***This photograph depicts an assistant demonstrating the equipment and its experimental attachment. The equipment is as follows: [a] NeuroScope™, [b] Powerlab™, [c] Laptop for Powerlab™ data acquisition, [d] Cannula in right antecubital fossa, [e] Foot electrodes, [f] Catheter assembly passed trans-nasally, [g] Finapress® blood pressure monitor, [h] skin conductance response electrodes, [i] Omni fuse™ infusion pump, [j] Digitimer™ electrical stimulator, [k] Computer-administered questionnaires, [l] Synectics Medical™ continuous pH recording device.***
(Photo courtesy of Abhi Sharma.)

2.15 Electrocardiograph (ECG)

Skin was firstly prepared by light excoriation (Nuprep® gel; Weaver and Co, Aurora Co, USA) to reduce impedance, electrodes (Ambu® blue sensor-P, Ballerup, Denmark) were subsequently placed in 3 areas; below the lateral aspects of the right and left clavicles and the left mid-clavicular line below the breast. A modified Einthoven's lead II electrocardiogram (ECG) was acquired at a rate of 2kHz using a commercial bio signals acquisition system (Powerlab™, AD instruments, Figure 38(a)) and monitored on the Neuroscope system.

2.16 Respiratory Monitoring

Respiration via a transducer (Braebon™ smart belt) placed around the lower chest recorded the in-line lung filling and chest inflation of the subject in real-time and was monitored on the Neuroscope system.

2.17 Study Procedure and Design

The specific study designs will be discussed in each chapter dealing with those results. What will be covered here is the experimental design and procedures, which were common to all studies performed. The five studies were performed over three years in three different locations in Denmark and the UK (Figure 39):

Study Time Schedule & Locations:

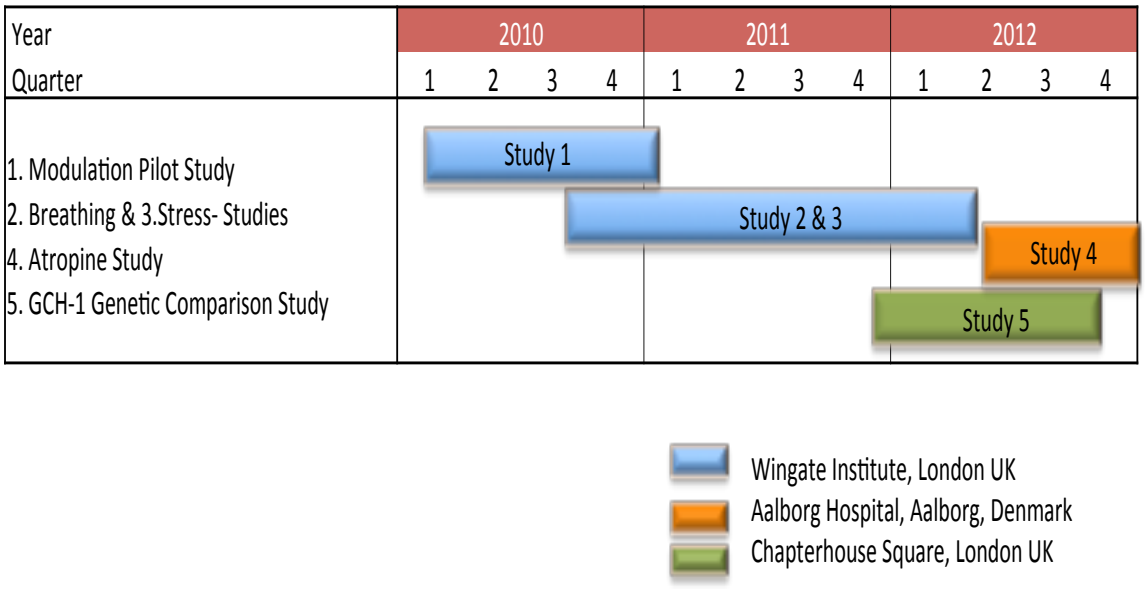


Figure 39 ***The time schedule of the five studies and there geographical locations.***

In order to conduct the experiment efficiently, three assistants were required (Figure 40):

- The first assistant performed the administration of the acid infusion; pH observations, electrical pain stimulation and recorded pain threshold response in each subject, and were blinded to all other data during the experiment.
- The second assistant was responsible for the ANS and skin conductance response (SCR) reading and recordings, as well as supervising the specific psychophysiological interventions, and was blinded to all other data during the experiment.
- ANS data analysis was conducted by the third person (analyser) who was not involved in the experiment, except for the administration of intravenous (IV) atropine or placebo. The third

assistant was blinded to the subject experimental and sensitisation status.

- Subjects were blinded to all data during the experiment and to their sensitisation status between studies.

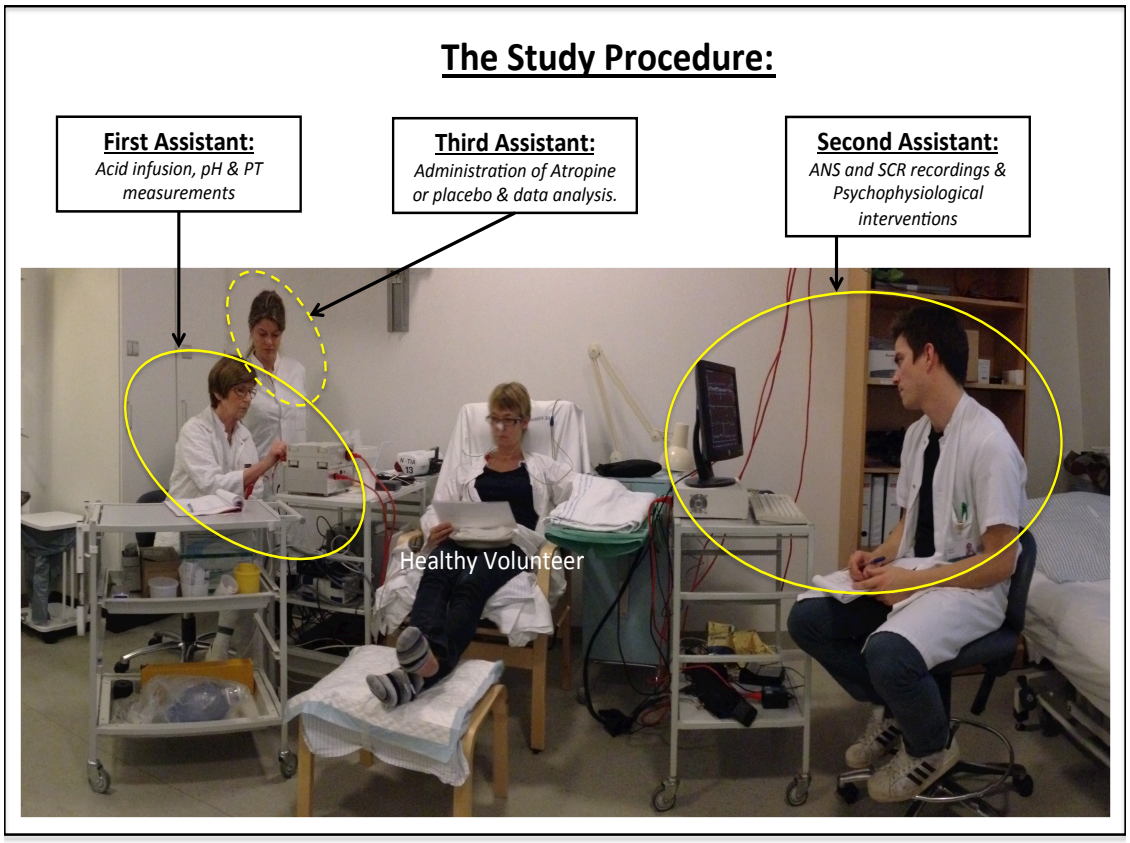


Figure 40 ***This photograph depicts the experimental setup, the healthy volunteer and the role of the three research assistants.***

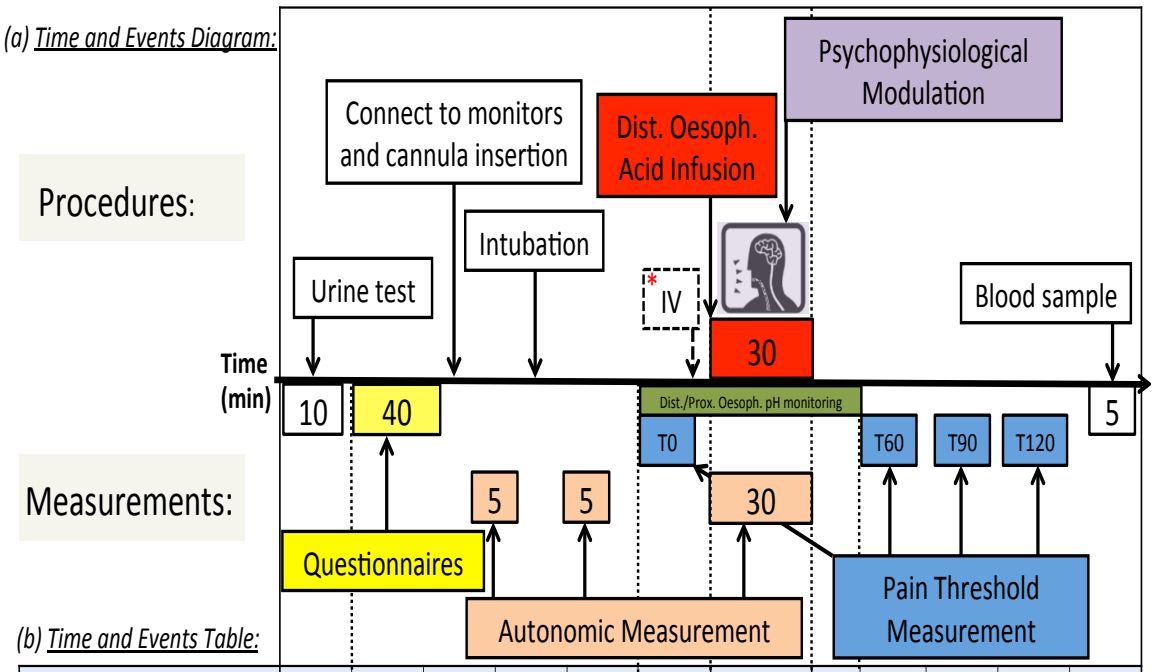
The study design was a double blind, randomised, crossover, prospective study, as the same recruited subjects were followed up throughout the duration of each specific study. The cohorts formed their own controls on subsequent visits by means of the cross over design. For the atropine study (study 4), there was a placebo control. Subjects were required to complete two to three visits, depending on the type of study, their sensitisation status and randomisation. For the pilot (study 1) and the

atropine (study 4) studies, the subjects first completed a screening visit, before being randomised. During the 'Breathing & Stress' study (studies 2 and 3), both sham (normal) and deep breathing protocols were randomly allocated directly after recruitment, and the non-sensitises (study 3) identified during the sham-breathing visit. All the subjects' subsequent crossover visits were undertaken a minimum of two weeks after the preceding visit. The studies therefore produced "paired data sets" in the majority of cases. To randomise the subjects without bias, approved statistical software was used (www.randomisation.com) in advance, and subjects were randomised in a "2 x 5 - block randomisation" pattern.

2.18 Experimental Design & Protocol

Subjects were asked to fast from midnight prior to the experiment. All experiments were started at 9 AM in the morning to compensate for, and rule out, HPA-axis diurnal variation fluctuations. There was no external interference during the duration of the experiment.

On the day urine pregnancy test and general health screening questionnaires and checks were completed prior to starting the experiment. This was to confirm that all inclusion and exclusion criteria were met. The same experimental design was used on all visits, with only the type of psychophysiological modulation altering (see Figure 41).



(b) Time and Events Table:

Time elapsed (min):	0	10	50	80	100	110	120	150	180	210	270	400
Time period:	10	40	30	15	5	20	30	30	30	30	30	5
Time with regards to Acid :	-120	-110	-70	-40	-25	-20	0	30	60	90	120	130
Informed Consent	x											
Demography	x											
Medical History	x											
Inclusion/Exclusion	x											
Physical Examination (incl. weight/Height)	x											
Pregnancy & Drugs of abuse u-test	x											
Questionnaires		x										
Connecting to monitors (and cannula insertion)			x									
Intubation				x		*						
Autonomic measurements			x		x		x----------x					
Dist./Prox. Oesoph. & Foot Pain Threshold						x			x	x	x	
Distal/Proximal Oesoph. pH Measurement						x----------x						
30 min Oesophageal Acid infusion							x----------x					
Psychophysiological modulation							x----------x					
Blood sample												x

* IV atropine or placebo – study 4

Figure 41 ***Detailed Time and Events (a) Diagram and (b) Table, showing the stages and approximate time durations in minutes of each part of the experimental protocol. The timing of the procedures is shown above the time line, and that of the measurements below. T0, illustrates the PT at baseline (i.e. pre acid infusion); T60, T90 & T120, illustrates the PT at 60, 90 & 120 minutes post acid infusion cessation, respectively.***

Studies were performed in healthy volunteers, as described in section 2.2, who contacted our department in response to a posted advertisement. Full informed consent was obtained. Subjects with any history of current or chronic gastrointestinal, neurological or psychiatric medical problems or taking any medication affecting GI, pain or neuropsychological

function were excluded. Subjects then completed a set of questionnaires answered directly on a computer specifically provided for this purpose. In female subjects experimental visits were arranged to coincide with the follicular phase of their menstrual cycle. Where this was not possible, visits were scheduled so that subsequent visits occurred during the same phase of their cycle as their initial visit.

All studies were performed in a sound and temperature controlled laboratory. Temperature of the room was adjusted between 21-25 degrees Celsius according to the subject's preference. The subject was sitting upright in a comfortable couch throughout the study at an 80° angle with their head fully supported. Patients were then attached to ECG electrodes to monitor autonomic parameters, blood pressure and a breathing belt. When a baseline recording of 5-minutes was completed, the nasogastric catheter assembly was placed into the oesophagus. After a further period of rest, a second "post intubation" baseline recording of 5-minutes was acquired. Where necessary, the intravenous cannula would now be placed.

The subject's baseline pain tolerance thresholds were then recorded for the proximal oesophagus, distal oesophagus and foot. Subjects were then connected to a syringe driver, which delivered the hydrochloric acid over a 30-minute period into the distal oesophagus. Subjects were asked to rate their subjective pain and unpleasantness on an 11 point visual analogue scale after completing the acid exposure. (Figure 32)

Depending on the study type and random allocation, during the acid exposure each subject was asked to complete or was coached through a psychophysiological modulation protocol, for the duration of the acid

exposure phase. This could include: 1. Normal screening protocol, 2. Sham breathing protocol, 3. Deep breathing protocol, 4. Deep breathing protocol with atropine or placebo 5. Isometric "hand-grip" test protocol, and 6. A "dichotomous listening" stress test protocol (see section 2.20). Then followed a 30-minute-rest period, after which pain thresholds were once again recorded at 60, 90 and 120 min after the start of the acid infusion. The last procedure was to obtain a 5ml blood sample for genetic analysis (study 5), which was immediately labelled and frozen down.

2.19 Use of Psychophysiological Modulation

In the following section is an explanation of the neurobiology and underling theory of the psychophysiological modulations used in this thesis:

2.19.1 Physiological role of Respiratory Sinus Arrhythmia

Respiratory Sinus Arrhythmia (RSA) is an intrinsic resting function of the cardiopulmonary system. It is an active physiological function that has its own biological roles. By matching alveolar ventilation and capillary perfusion (V/Q matching, see figure 42) throughout the respiration cycle RSA improves respiratory gas exchange efficiency. With increase in alveolar ventilation during inspiration (V), there is an increase in capillary perfusion (Q) due to a SNS mediated increased HR in order to facilitate blood-gas transport. This is better understood when contrasted with the inverse effect of RSA, which gives rise to alveolar dead space (wasted ventilation) and increased intrapulmonary shunt (ineffective perfusion). Hence during expiration the PNS outflow-mediated drop in HR facilitates alveolar gas exchange. This function of RSA is useful as it saves cardiac

and respiratory energy in resting animals and humans. (227) In mammals, the effectiveness of certain cardiac reflexes is markedly modified by respiration. Reductions in heart rate are evoked by brief stimuli applied to the arterial baro- and chemoreceptors (Figure 44), but only if they are applied during expiration. (228) Stimuli given during inspiration are less effective or totally ineffective, as this will only enhance the "dead space or shunt" phenomena (Inverse RSA). It can be predicted that any cardiac reflex would be modulated by respiration, since the preganglionic neurons themselves are under respiratory control, which is mediated by these neurons. In contrast, stimulation of receptors in the airways and cardiac C-fiber receptors all evoke reflex excitation of cardiac vagal outflow, potentially resulting in a bradycardia, which is modified by respiratory drive and is evident as RSA.

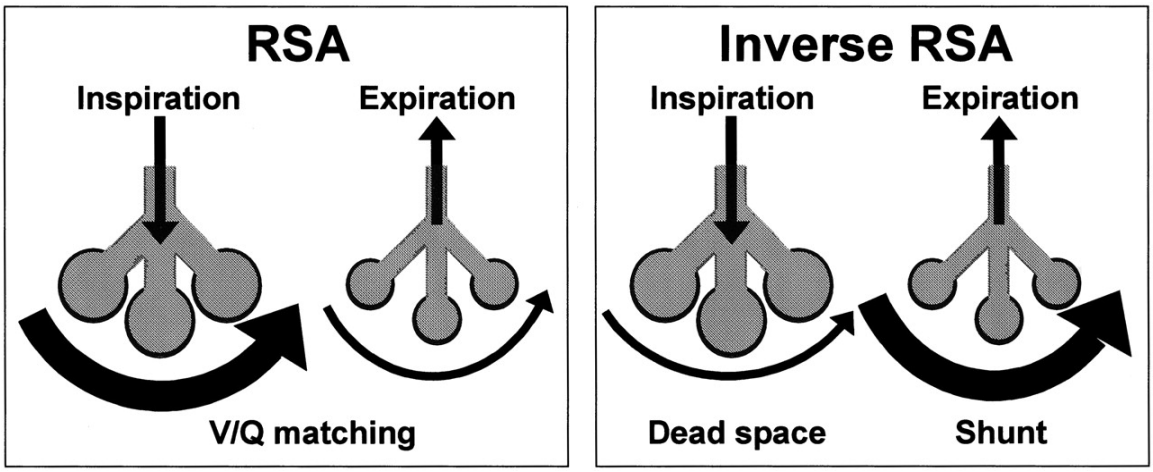


Figure 42 Schema showing the effects of RSA and its inversion (inverse RSA) on the relationship between alveolar gas volume and capillary blood flow during inspiration and expiration. Horizontal bows and vertical arrows indicate the volume of blood flow and the direction of gas flow, respectively. RSA improves respiratory gas exchange efficiency by matching alveolar ventilation and capillary perfusion throughout the respiratory cycle, while inverse RSA results in increased alveolar dead space (wasted ventilation) and increased intrapulmonary shunt. (Adapted from Hayano, 2003) (227)

Even though evidence indicates that RSA magnitude and cardiac vagal tone seems to be regulated separately, RSA's HF component of HRV is

ubiquitously used as an index of cardiac vagal function, and is intrinsically physiologically connected. (229)

2.19.2 RSA as a component of HRV

Short-term HRV measured as beat-to-beat variation of RR interval shows unique behaviours in response to stress and diseases. Most physiological parameters are kept constant around their own set points in the absence of external or internal turbulence/stressors. Hence states in which such constancy is lost could arguably be considered as indicative of disorders. Following this concept, RR interval is expected to be stable at rest (health) and to become unstable under distress (disease). However, the reverse is clinically observed. In Figure 43, RSA fluctuations of RR interval is most variable in healthy subjects at rest, and it reduces with mental and physical stresses, and is almost non-existent in patients with severe heart failure even at rest. This indicates that increased fluctuation of RR interval is a characteristic of health and is suppressed in distress and diseases. (227, 230, 231)

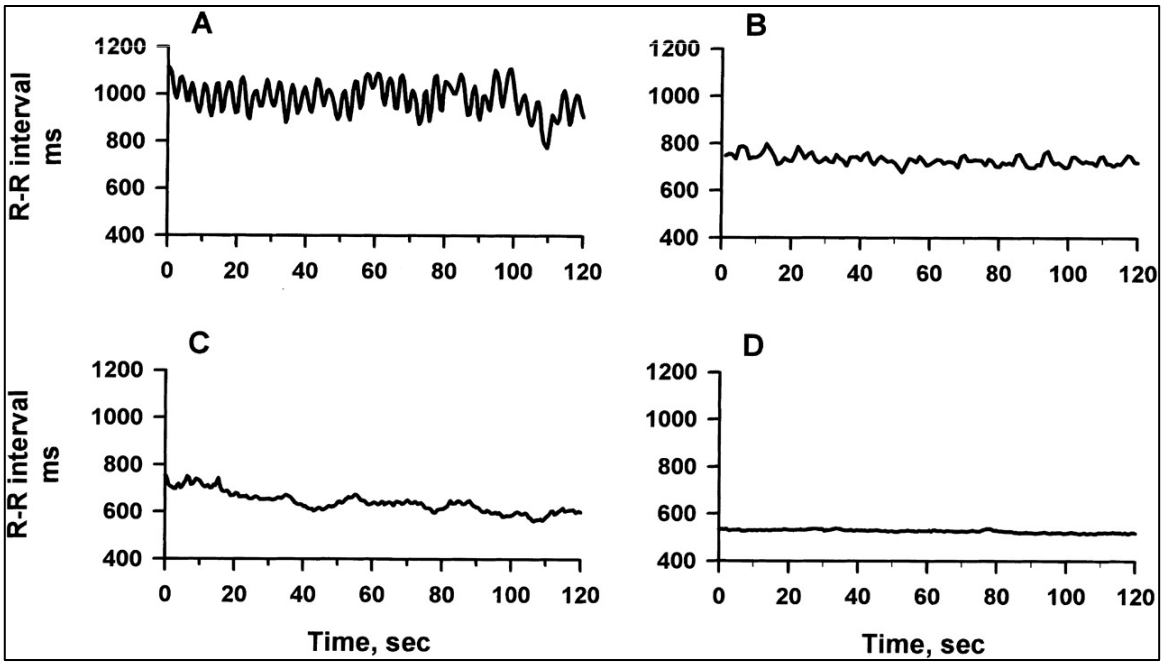


Figure 43 *Trendgrams showing fluctuation of beat-to-beat RR interval in various conditions. (A) RR intervals were measured from 2-min ECG in healthy young subjects during supine rest, (B) mental arithmetic stress testing, (C) and ergometer exercise testing and (D) in a patient with severe congestive heart failure at rest.*
(Adapted from Hayano, 2003) (227)

In short-term HRV such as those shown in Figure 43, RSA is the most prominent and consistent component. RSA is an oscillation of heart period in synchrony with respiration, which appears in power spectrum of RR interval as a peak within the so-called HF band (0.15–0.45 Hz, Figure 34) or, more appropriately, as a peak at respiratory frequency. Due to the difference in frequency characteristics of signal transfer between sympathetic and vagal modulation of heart rate, it is believed that the NA branch of the vagus solely mediates RSA. (232) RSA has been proposed and widely used as a quantitative index of cardiac vagal function, because the magnitude of RSA is attenuated with progressive suppression of cardiac vagal activity and abolished by complete vagal blockage with atropine. (212, 233-235) This is now controversial since improved understanding brought by theories of the divergent influences of different parts of the vagus nerve. (70)

2.19.3 RSA and Cardiorespiratory Control

Historically, the first references to RSA were made in the beginning of the 1900s. Wundt (204) stated that "*...respiratory movements are therefore regularly accompanied by fluctuations of the pulse, whose rapidity increases in inspiration and decreases in expiration.*" H.E. Hering (236) observed as early as 1910 that a functional relationship exists between the amplitude of RSA and the concept of vagal tone. He reported that, "*…breathing provides a functional test of vagal control of the heart.*" He when on to say "*…it is known with breathing that a demonstrable lowering of heart rate ... is indicative of the function of the vagi.*"

Presently it remains that central control of the cardiorespiratory system is complex and interactive. It is modulated by afferent inputs from areas in the mid- and forebrain such as the hypothalamus, amygdala and cortex, and is operated by means of a direct feed-forward control from the brainstem. (237) Emotional states and several routine behavioural responses like, for instance "the orientation" and "fight or flight" responses, causing marked variation in the HR. The amygdala receives projections from several nuclei involved in cardiovascular control that includes the hypothalamus, parabrachial nuclei, nucleus of the solitary tract (NTS), and dorsal motor column of the vagus. (238) The infralimbic and insular cortices are also linked and influence control. Consequently, it is a critical site for cardiovascular control and has the role of integrating the autonomic responses to emotional stimuli like fear, anger and stress. (237)

In the control of the ANS function the hypothalamus is a further key area, since it integrates information from somatic motor areas, emotional state and also humeral efferent activity. (238) It has connections to both sympathetic and parasympathetic control, as the hypothalamic nuclei

connects directly with the ventrolateral areas in the brainstem and the intermediolateral neurons of the cervical and thoracic medulla. (237) As a result of these rich midbrain and brainstem neuronal connections, cardiorespiratory control overlaps and is also intrinsic to the neurophysiological control, which mediates between emotional and physical states. This overlap forms the anatomical and physiological basis for a two-way regulation, where cardiorespiratory changes can influence emotional and autonomic states, and vice versa.

A physiological example of this is the observation that heart rate decreases during expiration. This occurs because HR is generated centrally by an inhibitory input from inspiratory neurons in the respiratory group projecting to the caudal ventral-posterior nucleus (CVPN) outside of the dorsal ventral nucleus (DVN) in the ventrolateral nucleus ambiguous (NA). (229)(Figure 44) As respiration-related fluctuations in the efferent pathway drives the inhibitory supply to the heart *via* the cardiac vagus, respiration is hence a physiological means of regulating RSA, and through its neurological connections gives a clear physiological window into modulating vagal outflow as reflected in the changes seen in PSD analysis. As such respiratory control effects cardio-autonomic regulation and has a psychophysiological effect, which proposes a mechanism for arbitrary modulation in laboratory conditions.

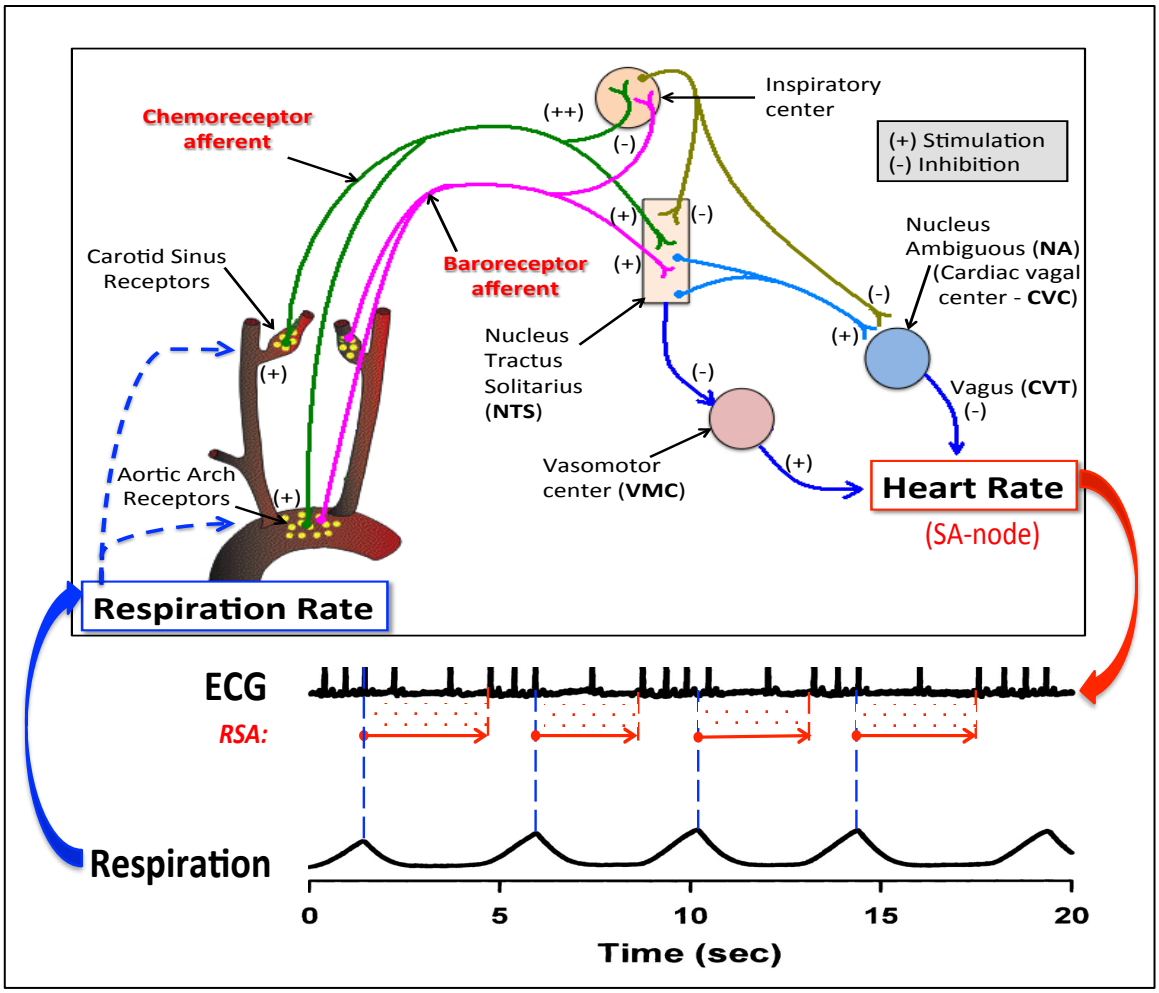


Figure 44 Diagram illustrating the pathways relating the interaction of cardiac and respiratory reflexes instrumental in producing RSA changes (illustrated by the red arrows). On expiration there is a lengthening in the RR interval, as seen on the ECG, due to reflexive vagal (CVT) inhibition. (Adapted from Daly, 1997) (228)

Applying this practically, it is observed that during isometric exercise and psychological stress, there is an increase in heart rate (and HRV) which is associated with an increase in the VLF frequency band, suggesting an increase in sympathetic dominance, compared to the baseline of healthy young subjects during supine rest. (Figure 45, upper red arrow) On the other hand during paced deep breathing the HR indicates exaggeration of RSA as observed in the HR trendgram and an increase in the 0.10Hz frequency band (HF) suggestive of parasympathetic dominance. (Figure 45, lower green arrow)(227, 235, 239) This supplies

strong neurophysiological support and justifies the use of these specific psychophysiological states as modulators that can be used to increase and decrease brainstem autonomic outflow.

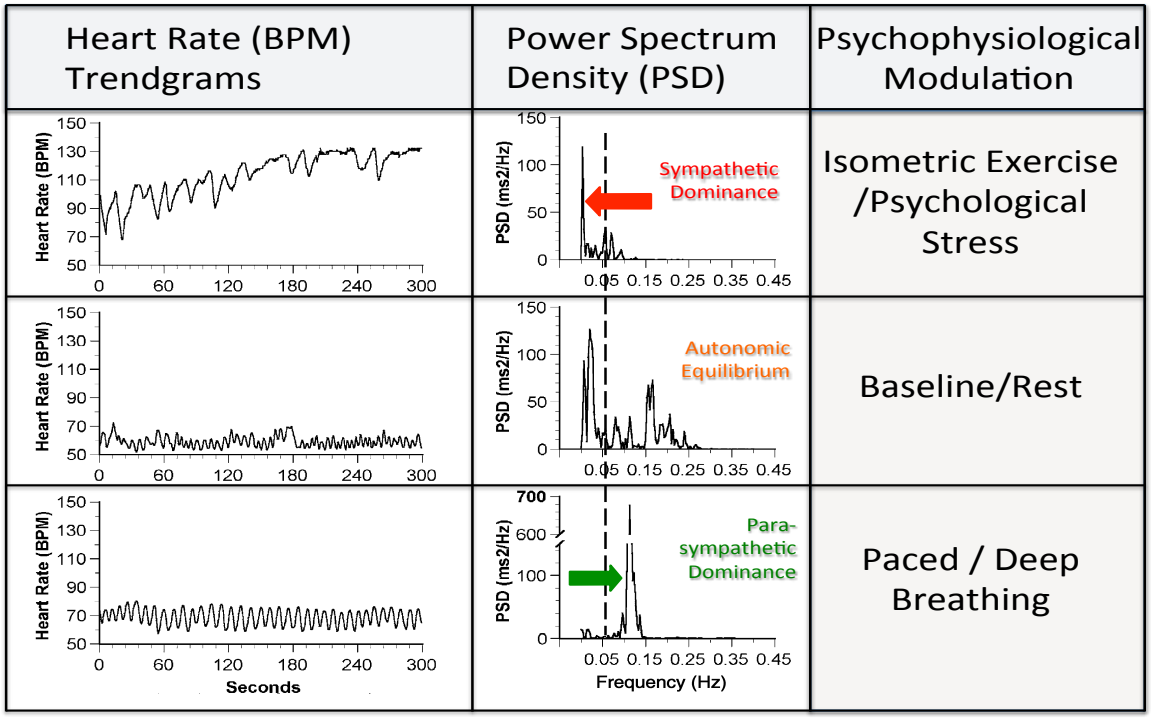


Figure 45 This diagram illustrates the effect of three different psychophysiological modulated states (column on the right) as compared with regards to Heart Rate (HR) Trendgrams (column on the left) and Heart Rate Variability Power Spectrum Density (PSD) (centre column): Isometric exercise and psychological stress (top row) has a gradual increase in HR and a VLF peak on PSD, suggestive of sympathetic dominance. Baseline supine rest in young healthy volunteers (middle row) has a responsive HR with RSA fluctuation. The PSD is balanced across VLF, LF & HF, suggestive of autonomic equilibrium. With paced deep breathing (bottom row), the HR trendgram indicates an exaggerated RSA pattern with HF peak on PSD. This would indicate an enhanced parasympathetic response. (Adapted from McCarthy, 2009) (213)

2.20 Psychophysiological Modulation Protocols

As discussed in section 2.19.3, the vasomotor centre (VMC) located in the medulla is vital to the maintenance of the autonomic tone and its activity is modulated by a number of psychological and physiological stimuli. As was seen (Figure 45), psychological distress and physical exercise increases the sympathetic tone while reciprocally decreasing

the parasympathetic tone. This increases the heart rate, cardiac output and blood pressure. In contrast, forced deep inspiration and expiration exaggerates the normal RSA regulated by the parasympathetic output of the brainstem vasomotor centre leading to a slowing of the heart rate. These physiological alterations in the autonomic tone therefore provide an excellent opportunity to modulate the ANS selectively and observe its effect on oesophageal sensitisation to acid. Hence the psychophysiological modulations that were used in this thesis were:

2.20.1 Screening visit protocol

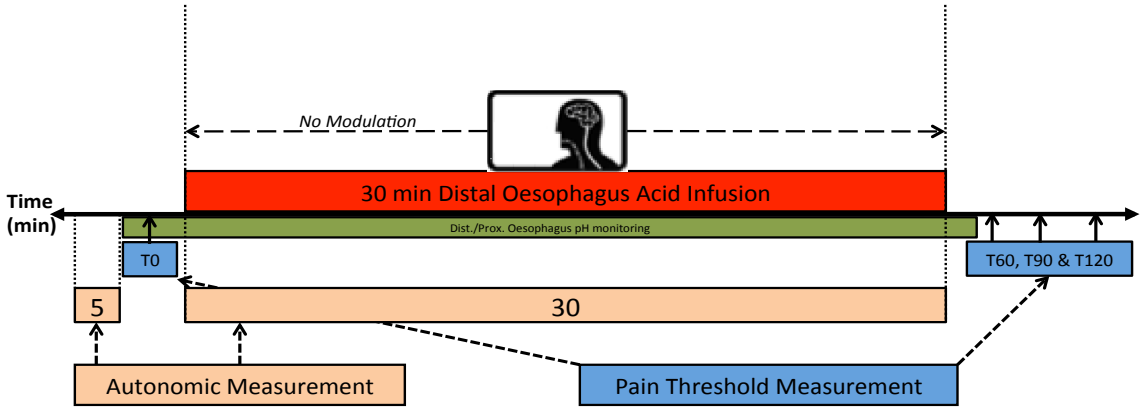


Figure 46 Diagram illustrating the psychophysiological modulation protocol for the screening visit. As this visit during study 1 was to serve as a baseline visit, no psychophysiological modulation was performed during the 30minitus acid infusion period (red bar). Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).

This protocol has no psychophysiological modulation component (Figure 46), and functioned primarily to provide a baseline for comparison, and to discriminate between sensitisers and non-sensitisers. Normal autonomic fluctuation patterns were observed, as illustrated in Figure 53 (panel 1).

2.20.2 Sham breathing protocol

This protocol was designed to simulate the cognitive distraction, interpersonal interaction and somatic focus components of the deep breathing protocol and in doing so, provide a psychological control intervention.

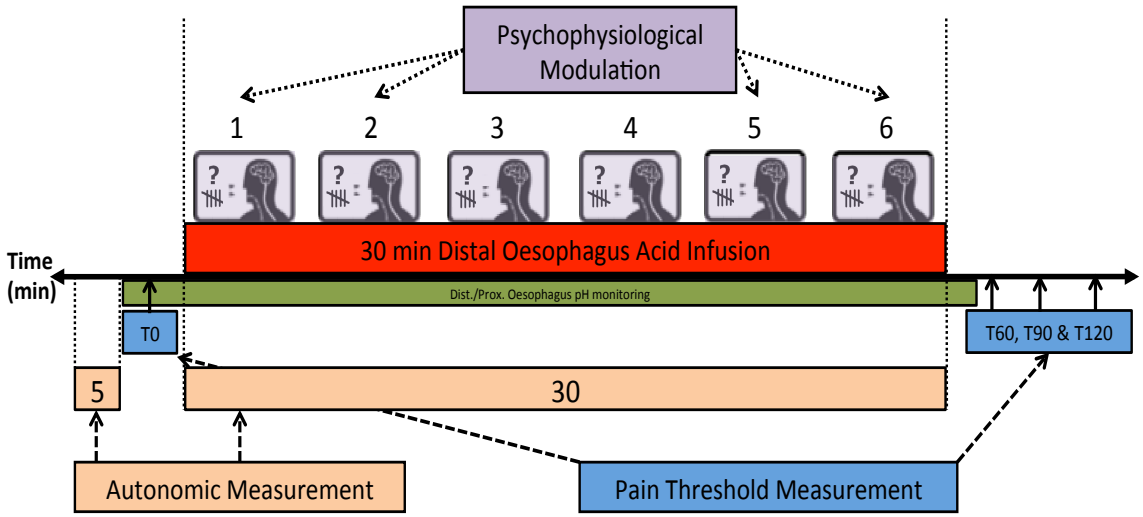


Figure 47 *Diagram illustrating the psychophysiological modulation protocol for the 'sham breathing' visit. The subject was asked to count 6 breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar). Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).*

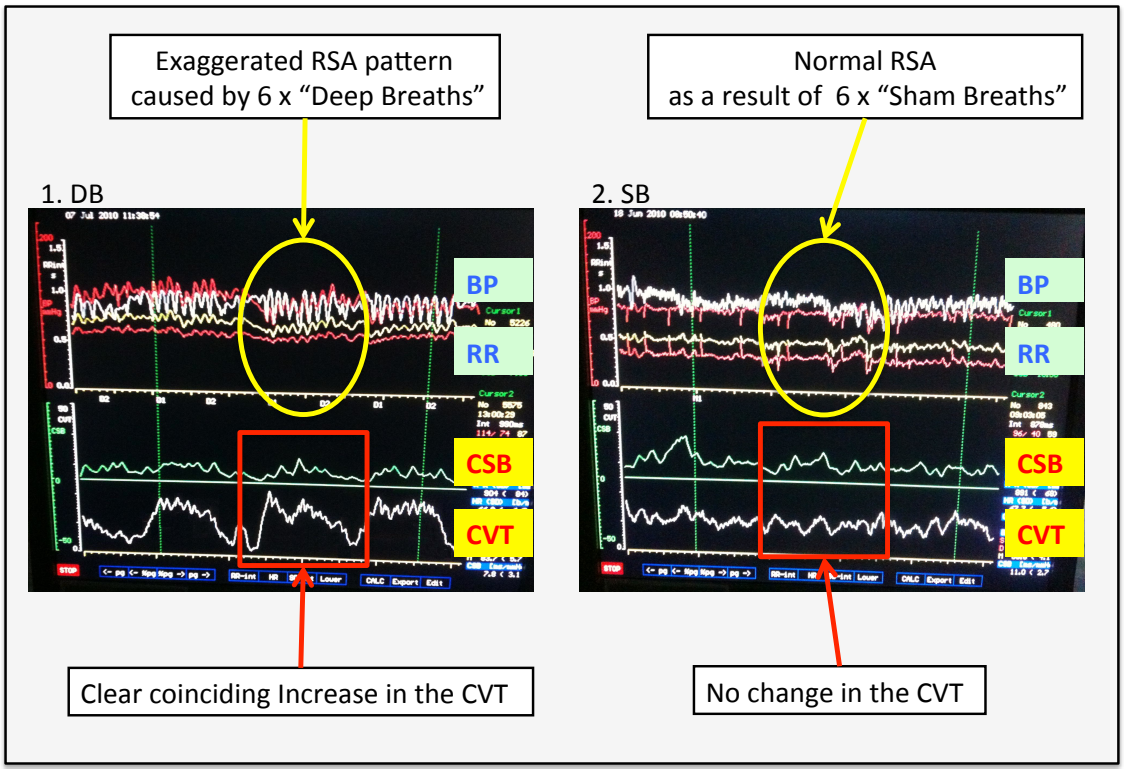


Figure 48 *This diagram shows two actual healthy volunteer NeuroScope™ 'screenshots'. The graphs in the upper half of each panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of etch panel shows the CSB (green graph) and CVT (white graph) each labelled as such. In panel 1.DB (left) the yellow oval highlights the RSA changes in the BP & RR, brought about by six consecutive breaths of the 'deep breathing protocol'. The red box below highlights the coinciding increase in CSB & CVT from baseline. Compared to this the yellow oval in panel 2.SB (right) highlights the normal RSA fluctuations in the BP & RR, brought about by six consecutive breathes of the 'sham breathing protocol'. Highlighted in the red box below, is normal CSB & CVT similar to that which is observed at baseline.*

Here the subject was asked to periodically focus and count six normal consecutive breaths, every five minutes, throughout the 30-minute acid infusion period. The subject was not given any specific instructions with regard to respiratory rate, depth or type (Figure 47). The intervention was not associated with any RSA changes and the respiration component of this intervention had thus no physiological effect, as illustrated in Figure 48 (panel 2).

2.20.3 Deep breathing protocol

The deep breathing protocol used in this study was based and modified from a procedure described by Roland D. Thijs *et al*. (240) They used the original "deep breathing through pursed lips" protocol, as a respiratory countermanoeuvre to maintain the blood pressure (BP) of patients diagnosed with orthostatic hypotension in context of autonomic failure. This manoeuvre was designed to activate the "respiratory pump" and affect the BP in several ways. It augments venous return when the intrathoracic pressure becomes more negative during inspiration and in doing so, stimulates the aortic arch and carotid baro- and chemoreceptors to increase afferent stimulation of the nucleus of the solitary tract (NTS) and VMC. (229)(Figure 44) It thereby increased the cardiac vagal outflow to the heart and blood vessels that is associated with synchronised augmentation of the RSA. (Figure 48, panel 1) They further demonstrated that patients who trained with BP biofeedback improved the effectiveness of the countermanoeuvres while fully preventing hyperventilation, and reproducibly could increase parasympathetic outflow in laboratory conditions. (210)

The enhanced parasympathetic outflow in this study was achieved by paced breathing at full inspiratory capacity in 4 sec, followed by exhaling to forced expiratory vital capacity in 6 sec. This was repeated at a frequency of 0.1Hz (6 breaths per minute), for a one-minute period. This manoeuvre was repeated every 5 minutes for the 30-minute duration of the acid infusion phase of the experiment, thus allowing for about 6 deep breathing cycles per 30-minute period (Figure 49). The amplified RSA and the coinciding increase in CSB and CVT, in contrast to the sham breathing protocol is illustrated in Figure 48, panel 1, as measured on the Neuroscope.

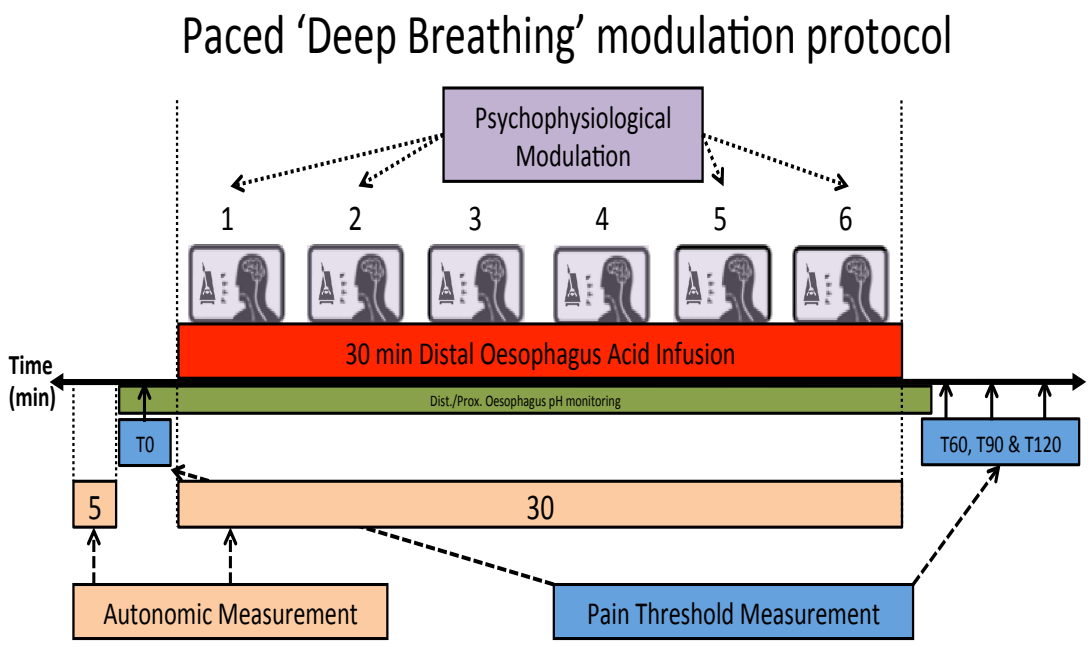


Figure 49 *Diagram illustrating the psychophysiological modulation protocol for the 'deep breathing' visit. The subject was paced to perform 6 deep breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar). Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).*

2.20.4 Deep breathing with atropine or placebo protocol

In both treatment arms of study 4, subjects were asked to perform the deep breathing protocol, as described in section 2.20.3, while they received the acid infusion. In one treatment arm subjects received IV saline (placebo) while in the other arm they received IV atropine in a double blind manner, given by the unblinded third assistant (see section 2.17). (Figure 50)

After baseline measurements of ANS and upper/lower oesophageal pain thresholds to electrical stimulation, the volunteers received a dose of 0.5mg atropine sulphate administered intravenously (IV) 5-minutes before the start of acid infusion (Figure 41). Its mechanism of blocking

parasympathetic tone has been shown to be pro-algesic in previous similarly performed studies. (241, 242)

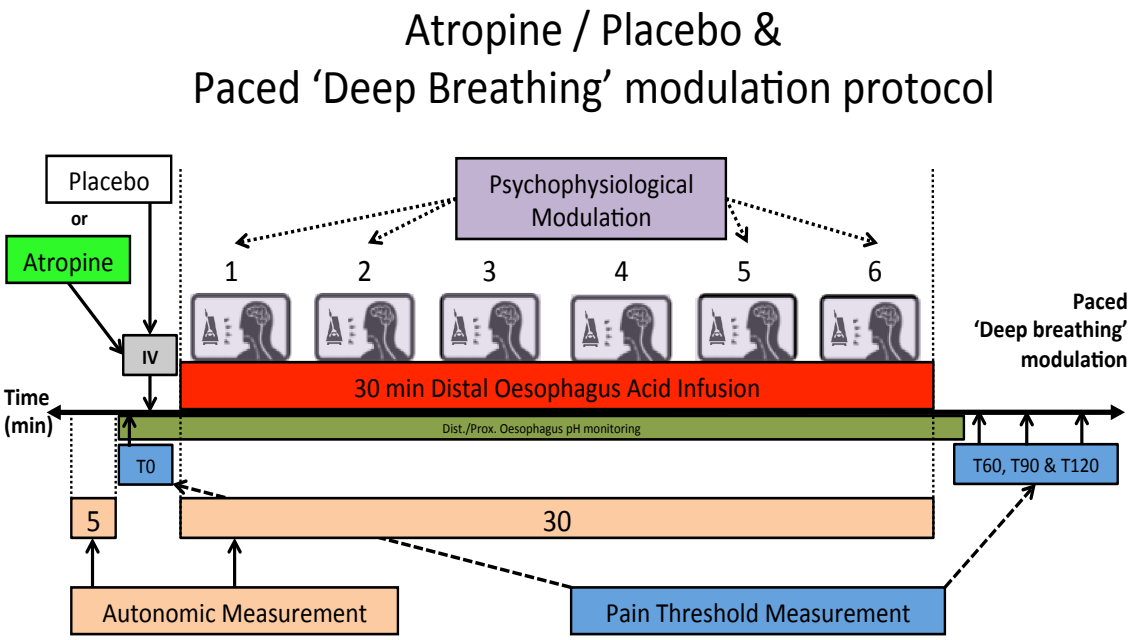


Figure 50 Diagram illustrating the psychophysiological modulation protocol for the atropine-placebo study. The subject was paced to perform 6 deep breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar) on all visits. Atropine or placebo was administered 5mins before the start of acid infusion. Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).

Atropine is a cholinergic (muscarinic) antagonist that in humans, at a low-dose, (≤ 2μg/kg IV) paradoxically decreases heart rate and increases RSA because of increased parasympathetic activity. At high doses (≥15μg/kg IV) atropine causes blockade of muscarinic receptors at the cardiac sinoatrial node and a marked reduction in parasympatholytic tone as seen by an increase in heart rate and decreased heart rate variability. This paradoxical response is not fully understood but atropine effectively antagonises the parasympathetic inputs to the SA node, therefore there is unopposed sympathetic activity,

which causes the increased heart rate. However it is thought that at low doses it doesn't cause this vasolytic function and instead causes a vagotonic reaction, which causes further bradycardia. (243) It has a half-life of approximately 4 hours, which covered the duration of the experiment adequately.

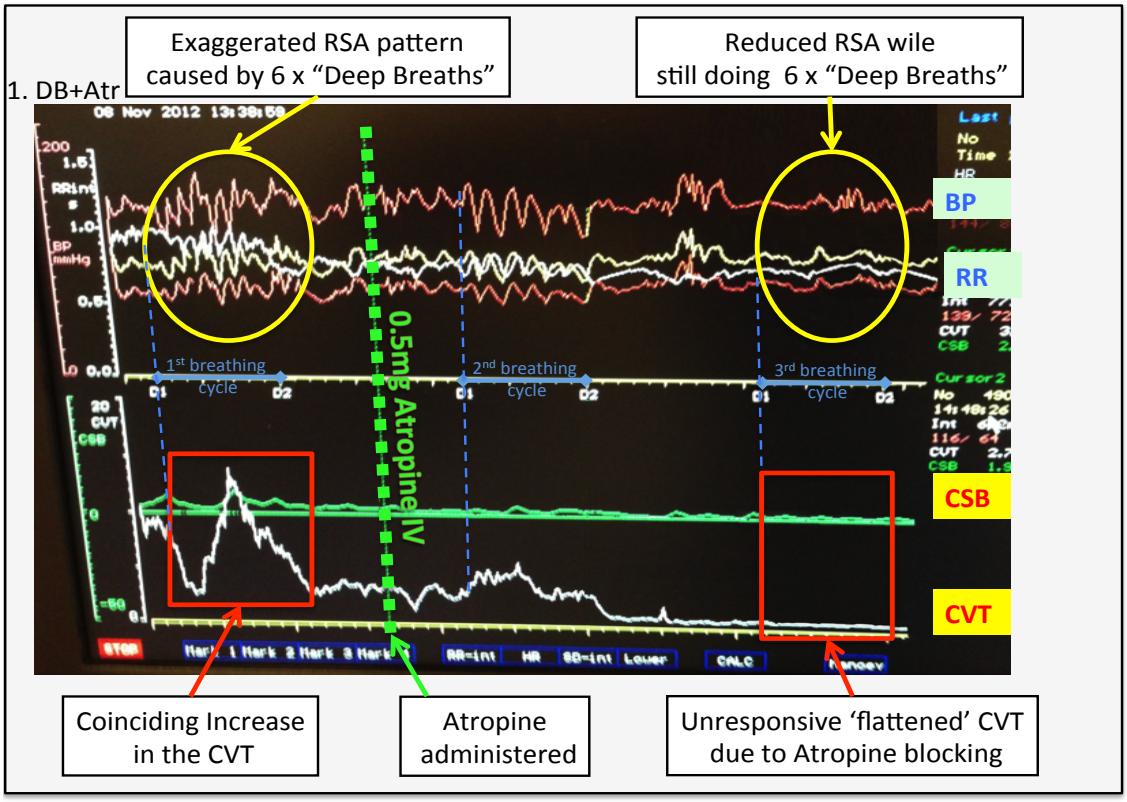


Figure 51 *This diagram shows 3 x deep breathing cycles (numbered in blue) of an actual healthy volunteer's NeuroScope™ 'screenshot'. The subject received 0.5mg Atropine IV between breath cycle 1 & 2 (green dashed line). The graphs in the upper half of the panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of the panel shows the CSB (green graph) and CVT (white graph) each labelled as such. The first yellow oval (left) highlights the RSA changes in the BP & RR, brought about by six consecutive breaths of the deep breathing protocol before the administration of the atropine. The red box below highlights the coinciding increase in CSB & CVT from baseline. Compared to this the second yellow oval (right) highlights reduced RSA changes in the BP & RR, indicating that even though the subject was doing six consecutive breaths of the deep breathing protocol the brainstem outflow is now reduced. The RSA, CSB & CVT is noticeably diminished by the second breath cycle, and almost totally unresponsive by the third. The red box on the right highlights the total block of the coinciding CSB & CVT response by atropine.*

In study 4, largely due to regulatory concerns over cardiovascular safety, a standard dose of 0.5mg of atropine sulphate IV was chosen and was used in accordance with the indications and dosing guidelines of the British National Formulary. This equates to approximately 7μg/kg. The methodological requirement was for a marked reduction in the RSA due to the antagonism of the parasympathetic outflow, in spite of implementing an effective deep breathing protocol. At this dose however there is not a marked tachycardic effect expected, which would allow for the active agent to remain blinded with regards to the participating subject. As a result of atropinisation the CSB and CVT will remain unresponsive during deep breathing, as illustrated in Figure 51. The increasing blockade of atropine is demonstrated in comparing the progression of three breathing cycles, one pre- and two post- atropine administration, and would thus be observable by the second assistant. (See 2.17, page 110.)

2.20.5 Isometric "handgrip" exercise test protocol

Isometric exercise stimulates the vasomotor centre located in the medulla and thereby increases the sympathetic tone while reciprocally decreasing the parasympathetic tone. This increases the heart rate, cardiac output and blood pressure. The protocol's aim was to examine the effect of enhancing the sympathetic tone in previously non-sensitising individuals to oesophageal acid sensitisation as identified by the screening visit. This was used to explore if an increase in sympathetic tone has any effect on subject's vulnerability to sensitisation, and explores the effects of physical stress on pain sensitisation.

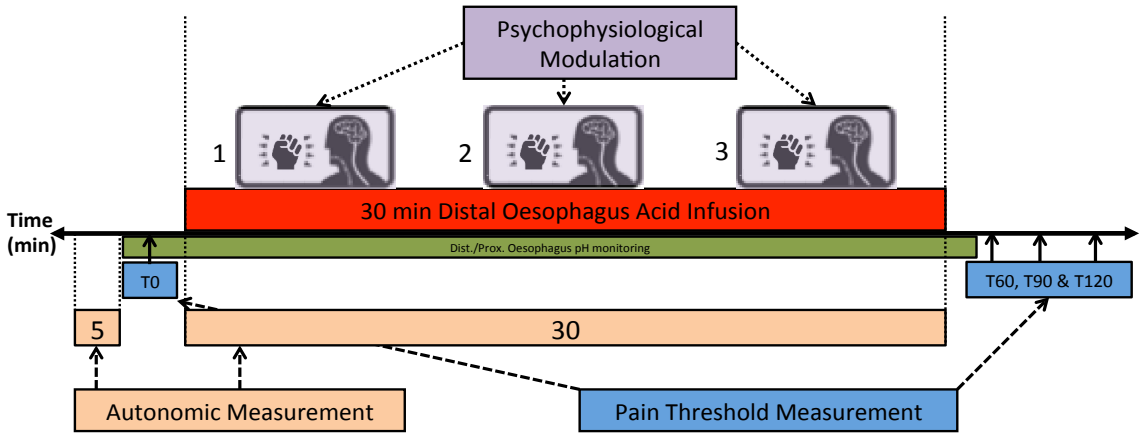


Figure 52 Diagram illustrating the psychophysiological modulation protocol for the 'handgrip' visit. The subjects were directed to complete three separate isometric handgrips sustained for 5mins (purple figures) during the 30minitus acid infusion period (red bar). Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).

Sympathetic tone was increased with isometric exercise using a specifically designed handgrip equipped with a 'power feedback' meter. To standardise the force applied by selected subjects in this study, 30% of the maximal force possible was applied and maintained over five minutes. (162, 244, 245) This physiological modulation was then repeated three times during the 30-minute acid infusion. What is experimentally observed is a gradual increase in the BP and heart rate (decrease in RR-interval) over the 5-minute period. (Figure 52) This is associated with a gradual decrease of both the CSB and CVT, as illustrated in Figure 53, panel 2.

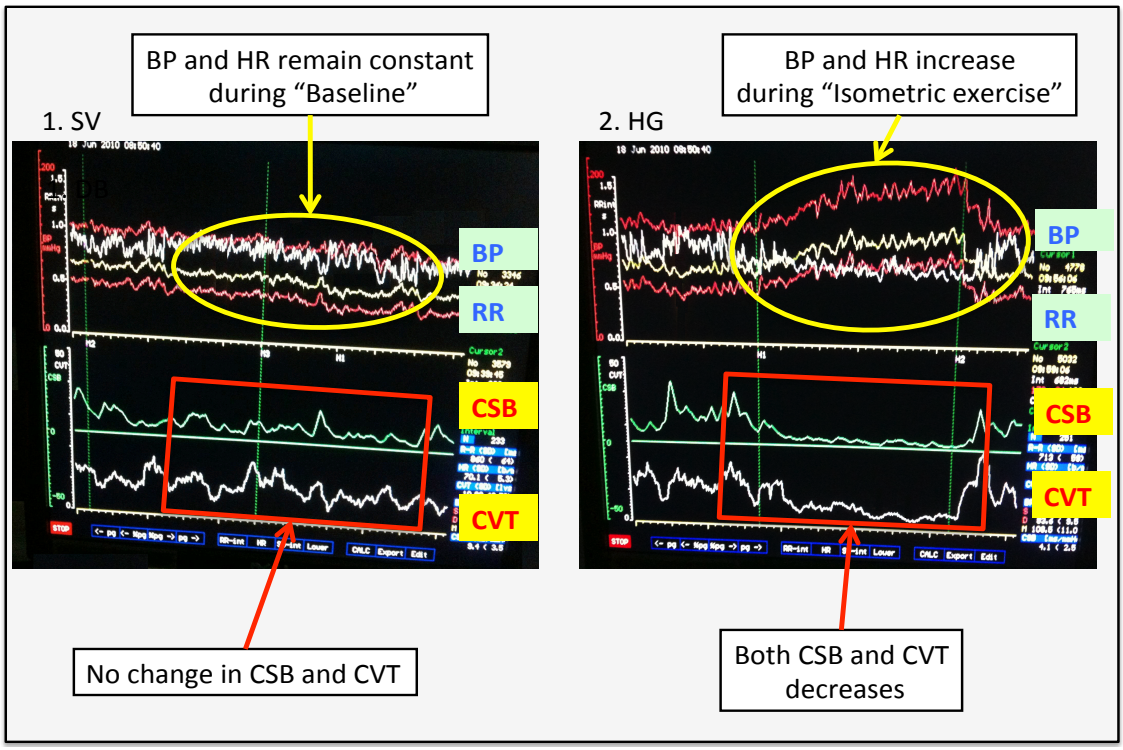


Figure 53 This diagram shows two actual healthy volunteer NeuroScope™ 'screenshots'. The graphs in the upper half of each panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of each panel shows the CSB (green graph) and CVT (white graph) each labelled as such. Panel 1.SV (left) is a recording during the 'screening visit' protocol. The yellow oval highlights normal RSA fluctuations in the BP & RR during baseline recording. Similarly, highlighted in the red box below, is normal CSB & CVT observed at baseline. Compared to this the yellow oval in panel 2.HG (right) highlights a gradual increase in BP, with a decrease in RR, brought about buy the 'Handgrip protocol'. Highlighted in the red box below, is the coinciding decrease in CSB & CVT.

2.20.6 "Dichotomous listening" psychological stress test protocol

Psychological stress increases the sympathetic tone while reciprocally decreasing the parasympathetic tone. This increases the heart rate, cardiac output and blood pressure. Psychological stress induction was achieved by using dichotic listening, which involves two conflicting types of music delivered simultaneously at 30 dB, via separate headphone channels. The subject heard Folk music in a foreign language in one ear and "heavy metal" music in the other ear. All subjects were exposed to the same pre-recorded music selection. This technique, which has been

previously validated by other investigators, has also been used to examine the impact of stress on visceral perception in patients with irritable bowel syndrome. (246-248) Following the results of study 1, to increase the subjective degree of psychological stress induction, subjects in study 3 were also asked to perform a standardised reading and mental arithmetic task while listening to the dichotomous music tract. This adaption was used to further increase the degree of psychological stress induction and has been validated by our group in inflammatory bowel disease (IBD) studies. (249)

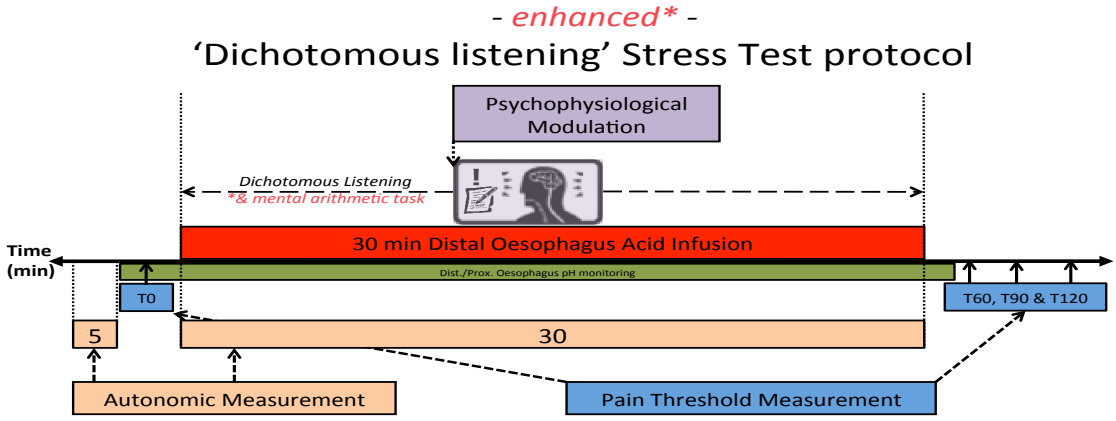


Figure 54 *Diagram illustrating the psychophysiological modulation protocol for the 'stress test' visit. Subjects listened to a conflicting duel track sound recording (purple figures) during the 30minitus acid infusion period (red bar). During study 3, it was augmented to include a mental arithmetic task as well. Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).*

2.21 Data handling and statistical analysis

All statistical analyses were performed using proprietary software (SPSS® v.19, IBM Inc., USA, Excel® Microsoft Inc., California USA & Prism® v.6.0c GraphPad Software Inc., California USA, Stata® V10.0, Stata Corp., Texas USA) in consultation with an accredited bio-statistician. Analysis included:

2.21.1 Primary endpoint analysis

In healthy volunteers an absolute fall in pain threshold (Δ Avr PT) of ≥6mA from baseline in the distal oesophageal following acidification was required for sensitisation to be documented as previously reported in the model (51, 191). Changes in PT were analysed using linear mixed effects regression models with maximum restricted likelihood (fixed effects: time, interventions i.e. deep breathing/sham breathing; atropine/placebo; random effect = subject) with T0 thresholds accounted for in the model as zero to yield a regression coefficient for intervention effect (with confidence interval (CI)). In study 2 & 3 the trapezoid area-under-the-curve (AUC) was calculated for all subjects at each time point for oesophageal pain thresholds. Comparisons of AUCs were undertaken with Mann-Whitney U test (pilot-&-study 2) and a repeated measures analysis of variance (ANOVA) with appropriate correction for multiple testing. Comparisons between groups were made using either the Student's t-test if parametric, or the Wilcoxon signed ranks test if non-parametric (pilot study). Comparisons between unpaired groups were made with an unpaired t-test or Mann-Whitney U-test depending on distributional assumption. All tests were two-tailed, and paired (same group) and non-paired (inter group) t-tests were used. All confidence intervals are given to 95% and p value significance was taken at $p<0.05$.

For the autonomic measures, normality of distribution was tested with histograms for each data set and was parametrically distributed. Analysis of Variance (ANOVA) was used to determine the measures of effect of ANS regulation for the differing modulations over multiple time points. For pain thresholds, the change in threshold from baseline was calculated for each time point and averaged to give a mean change in threshold for each individual subject. (250) The mean change of differing modulations across all time points was analysed using multivariate

analysis of variance (MANOVA). Baseline threshold was accounted for in the model. It allowed for repeated measures within patients, and for missing data. The residual variance from the model was used to calculate 95% confidence intervals for the differences in least square means between groups, and p value significance was taken at $p<0.05$. Distributional assumptions underlying this analysis were assessed by inspection of residual plots. Homogeneity of variance was assessed by plotting the residuals against the predicted values from the model, whilst normality was assessed by the use of normal probability plots.

2.21.2 Secondary endpoint analysis

To identify the factors associated with the magnitude of sensitisation, simple linear regression was used to determine the relationship between groups. Regression analyses on subgroups were performed if a relationship was present. Pearson's 'product-moment coefficient' was used for the correlations. The Bonferroni correction was used during multiple comparisons for all illustrated correlations. For some of the minor observational correlations, where the correction was not applied, it was clearly stated. Confidence intervals are given to 95% and p value significance was taken at $p<0.05$.

2.21.3 Sample size power calculation

The primary endpoint of these studies was the utility of psychophysiological manipulation of ANS in modulating oesophageal VPH in healthy volunteers who sensitise to acid. Calculation of sample size was done from this endpoint. The sample size calculation on the large amount of data we have over our research group's 5-year experience of manipulating the oesophageal VPH. (184-190) The group has produced summary data from these trials that show the mean effect

of placebo and acid in reducing sensory thresholds to electrical stimulation at 30 and 60 minutes as well as the mean and standard deviations of responses.

For studies 2 and 3, a 7.2mA was used as the between-subject standard deviation of the two groups (acid plus breath), and we estimated the within-subject standard deviation for both groups as 9.3mA based on previous studies using the acid induction VPH model. (175) Using a mean value of 83.4mA for the control group, and the within-subject standard deviation of 9.3mA in order to achieve 6mA difference between the two groups at 5% significant level and 80% power (beta of 0.8 p=0.05), the minimum sample size was calculated to be 30 by using the paired t-test. That means a total of 30 subjects will enter this paired designed study. The probability is 80% that the study will detect a treatment difference at a two-sided 5% significance level, if the true difference between the treatments is 5 units. This is based on an assumption that the standard deviation of the response variable is 9.33mA as in previous studies. On average only about 64% of recruited subjects who will enter the study will sensitise to acid, as such, it was necessary to recruit 47 subjects presuming a dropout rate of zero, and 53 subjects assuming a 10% drop out rate.

For study 4, the primary endpoint was the utility of physiological manipulation of ANS in conjunction with atropine in oesophageal VPH in healthy volunteers who sensitise to acid with a ≥6mA difference between the two groups. Hitherto this has not been studied so it was impossible to estimate the size of any effect accurately, so it was based on previous studies using atropine blockade. (251) Calculation of sample size for placebo vs. atropine was used, with a 30% reduction in sensitisation from baseline with atropine or saline (+/- 2 SDs) with a beta of 0.8 p=0.05,

giving n=7 in each treatment arm, and a sample size of 14 sensitisers. Based on previous studies using this model, a conservative estimated non-sensitiser rate of 40%, with a dropout rate of 10%, was used to back-calculate a minimum-screening cohort of 30 subjects.

3 Effect of Psychophysiological Modulation on Acid Induced Oesophageal Hypersensitivity - Pilot Study

3.1 Introduction

Psychiatric comorbidity is common in FGID. It is widely observed that IBS patients have a greater degree of anxiety and depression than either healthy controls or patients with inflammatory bowel disease. (252) A similar finding is seen in other medical conditions like Non Cardiac Chest Pain (NCCP) and patients diagnosed with coronary artery disease. (253, 254) The lack of clearly identifiable biological markers along with the aforementioned associations promotes the belief that these disorders are clinical manifestations of psychosomatic disturbance. Exploratory findings like the enhanced perceptual responses to experimental gut stimulation demonstrated in FGID patients, further strengthens the likelihood of this hypothesis. (152) Visceral pain hypersensitivity (VPH), clinically presents as hyperalgesia and allodynia, and occurs because of peripheral and central sensitisation, presently understood to result from the upregulation of nociceptive pathways. Given that both psychiatric comorbidity and VPH are common findings in FGID, an important question now being raised is how they interact in the pathophysiology of these disorders.

Persisting IBS symptoms develop as a result of gastroenteritis in about 30% of individuals (Post-infectious IBS (PI-IBS)), suggests that there is a link between inflammation, gastrointestinal injury and subsequent sensory dysfunction. (40) Furthermore it has been observed that the likelihood of developing PI-IBS in patients who were hospitalised when they

experienced gastroenteritis is much higher in those patients also suffering with comorbid anxiety, suggesting that psychiatric factors may modulate this link, although the exact mechanisms remain unclear. (255) A preceding history of inflammation or injury leading to somatic and visceral pain syndromes is also commonly seen in a variety of medical conditions, and includes cases of PI-IBS (40), Post-Herpetic Neuralgia (256) and also NCCP where acid reflux is a common finding. (257) There however remains great individual variety in the susceptibility for developing these post insult chronic conditions, as for instance only about a third of patients with gastroenteritis go on to develop PI-IBS. (40)

It is now clear that in the gastrointestinal tract inflammatory and immune mediators can facilitate peripheral afferent nociception (Peripheral Sensitisation) and subsequently upregulate nociception at or above spinal dorsal horn level (Central Sensitisation). (152) In "post insult" affected tissues, peripheral and central sensitisation manifests clinically with heightened sensitivity to experimental stimuli (hyperalgesia or allodynia). (152) This interface between gut lumen, sensory-neural pathways and the higher brain centres is closely regulated by the Autonomic Nervous System (ANS). (152) The degree of regulation suggests that altering ANS balance modulates bowel sensitivity, for example in healthy volunteers greater colonic sensitivity to balloon distension, has been shown by increasing sympathetic nervous system dominance. (153)

In patients with gastro-oesophageal reflux disease experimental oesophageal acidification is associated with enhanced sympathetic dominance (154), while in NCCP patients reporting pain during acid infusion, a reduction in vagal activity was observed. (155) Sympathetically mediated mechanisms are implicated in several chronic

pain syndromes (258, 259) and are associated with diarrhoea predominant symptoms in FGID patients. (260) Both animal and human data support a vagally mediated inhibition of visceral nociception (181, 261), and constipation predominant symptoms in FGID patients. (61, 262) These observations provide a mechanism whereby psychological abnormalities via their influence on the ANS, could be translated into differences in transit and pain discrimination leading to clinical syndromes observed. The potential role of autonomic dysfunction in FGIDs is made more plausible by the report from the Mayo Clinic of eight patients with acute autonomic neuropathies who presented with apparently typical IBS symptoms. (263)

In the model of acid-induced oesophageal pain hypersensitivity, while most subjects demonstrate reproducible sensitisation to repeated infusions, a proportion do not sensitise at all. (53, 175, 191) In addition, there is inter-individual variability in the magnitude of sensitisation to the order of 23.8 ±12.8%(SD). (264) The reasons for this inter-individual variation in developing hypersensitivity remain unknown. Data presented by Sharma *et al.*, (179, 185) suggested that psychological trait factors such as anxiety and neuroticism, and physiological (ANS) arousal states (HR, MBP and CVT) correlated with the degree of acid-induced oesophageal sensitisation in this model. What remains still unknown is to what degree the modulation of these psychophysiological factors via the ANS, can affect the degree of acid-induced oesophageal pain. The combined study of these factors as well as the effect of their modulation will further enhance our understanding and improve our ability to identify the phenotypes predisposed to or protected against pain hypersensitivity in this model, by means of more effective "psychophysiological profiling". This in turn could have important implications for

understanding the development of visceral sensitisation in clinical states, and may also offer novel therapeutic possibilities.

The aim of the study was thus to determine the effects of psychophysiological modulation of the ANS on acid-induced oesophageal pain hypersensitivity and to ascertain if inter-individual differences in the degree of sensitisation were predicted by inter-individual differences to different ANS modulation types. It was hypothesised that sensitisation as expressed by the difference in average pain threshold (ΔPT) would be directly proportional to sympathetic nervous system activation (SNS: ΔSCR), and parasympathetic nervous system withdrawal (PNS: ΔCVT), as induced or amplified by different psychophysiological modulations. A secondary aim of the study was to expand on the data in order to determine whether psychological state and trait factors predicted the degree of sensitisation to acid in the model.

3.2 Materials and Methods

3.2.1 Ethics Committee Approval

All protocols for this study were submitted and approved by the University Senate Ethics Committee, 'East London and The City Research Ethics Committee - Alpha' (ref: 09/H0704/71). See section 2.1(page 77).

3.2.1 Subjects

20 healthy asymptomatic adult male and female volunteers, aged 18 to 50, were recruited by advertisement. Screening for acceptability for inclusion and exclusion criteria was completed as described in section 2.2 (page 77).

3.2.2 Oesophageal Manometry

For this study standardised oesophageal manometry (183) was performed in the first five subjects to determine the positions of the upper and lower oesophageal sphincter (UOS and LOS) from the nostril. As the LOS positions on these first five subjects were found to be accurate enough for the purpose of this study, only the 'pH change' pull through technique as described in section 2.3 (page 78), was used for the remaining 15 subjects.

3.2.3 Other Methods of Measurement

All other methods of measurement; Catheter Assembly (section 2.4, page 78), Oesophageal acid infusion (section 2.4, page 78), Oesophageal pH monitoring (section 2.6, page 80), Pain Threshold Measurements (section 2.8, page 82), Psychological assessment (section 2.11, page 85), Measurement of the Autonomic Nervous System (section 2.12, page 86) and Respiratory Monitoring (section 2.16, page 99), was performed as described in their specific sections.

3.2.2 Methods of Psychophysiological Modulation

For this study the Screening visit protocol (section 2.20.1, page 111), Deep breathing protocol (section 2.20.3, page 114) and Isometric "hand grip" exercise test protocol (section 2.20.5, page 118), was used as described in the specific sections, and illustrated in figure 3.1 below. The Psychological stress induction was achieved by using the "Dichotomous listening" psychological stress test protocol as described in section 2.20.6 (page 119), but without the subsequent "standardised reading and mental arithmetic task" adaption, which was introduced for study 3.

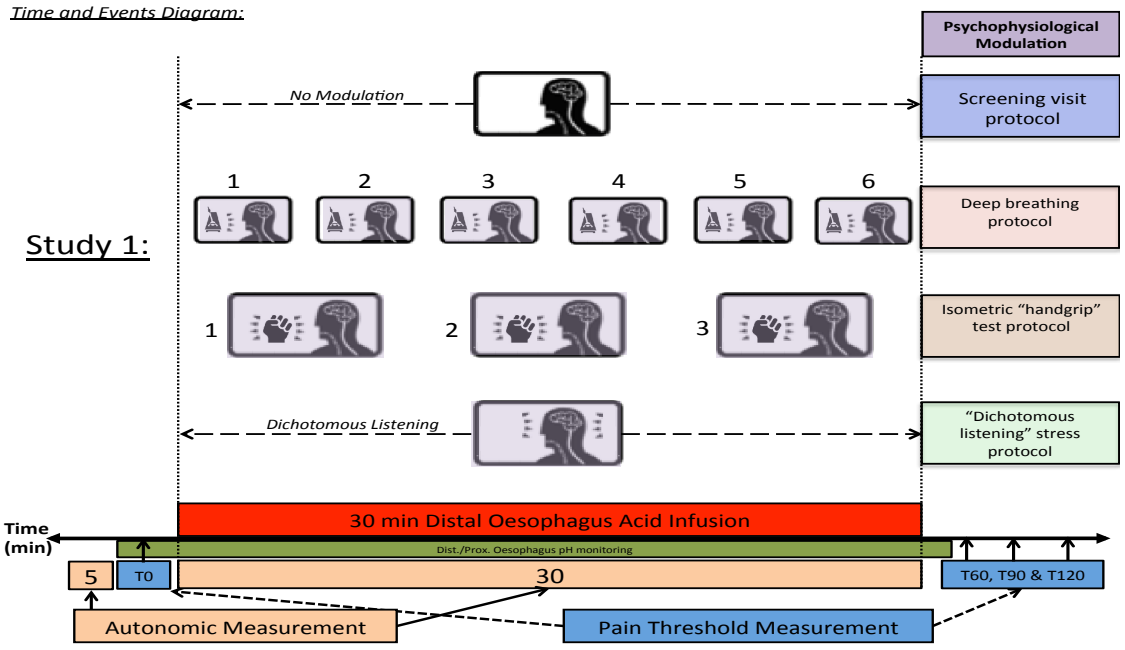


Figure 55 Diagram illustrating the psychophysiological modulation protocol for (from top to bottom) the Screening visit: As this visit during study 1 was to serve as a baseline visit, no psychophysiological modulation was performed during the 30minitus acid infusion period (red bar). 'Deep breathing' visit: The subject was paced to perform 6 deep breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar). 'Handgrip' visit: The subjects were directed to complete three separate isometric handgrips sustained for 5mins (purple figures) during the 30minitus acid infusion period (red bar). 'Stress test' visit: Subjects listened to a conflicting duel track sound recording (purple figures) during the 30minitus acid infusion period (red bar). Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).

3.2.3 Study Procedure, Experimental Design & Protocol

The experimental study design was that of a prospective pseudo-randomised three-tiered crossover double-blinded longitudinal cohort study. (Figure 56) The study procedure was followed as described in section 2.17 (page 99), i.e. using the 'three research assistants' method. The experimental protocol was used as described in section 2.20.5 (page 118), with 'time and events' proceeding as outlined in figure 40 (page 100). Specific modulation protocols were followed as discussed above in section 3.2.2.

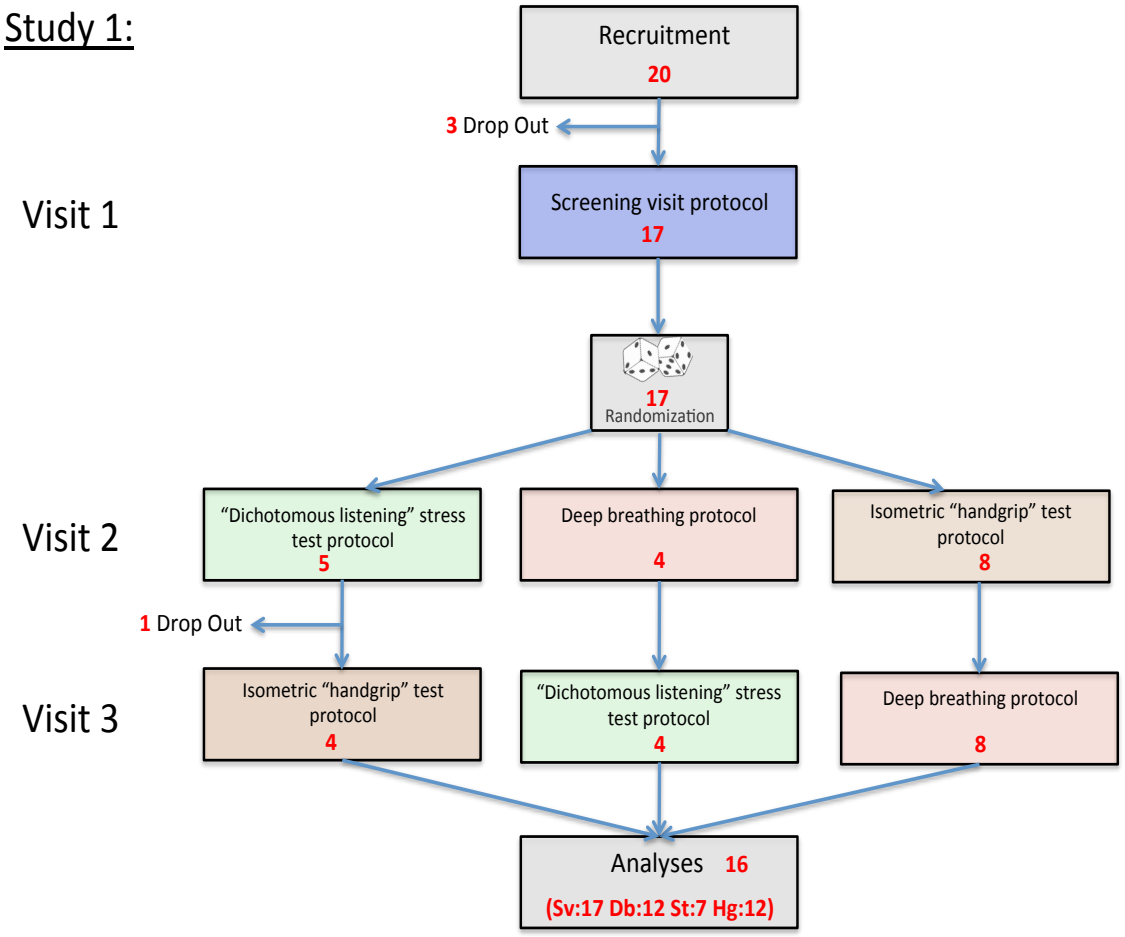


Figure 56 Flowchart illustrating the final numeric outcome of participants in the pilot modulation study (study 1). The experimental study design was that of a prospective pseudo-randomised three-tiered crossover double-blinded longitudinal cohort study. [Sv: Screening Visit, ST: Stress Test, Db: Deep Breathing, Hg: Hand Grip.]

3.2.4 Data Handling and Analysis

Demographic, pain threshold and autonomic data were normally distributed hence data are presented as mean ± SD, with parametric analysis. For the Isometric "hand grip" test protocol, 'collection bin' analysis (figure 66, page 152), and the "Dichotomous" psychological stress test, pain threshold 'pre and post acid' analysis (figure 60, page 136). All statistical analysis was completed as described in section 2.21 (page 120).

3.3 Results

During acid infusion, pH fell to <2.0 in the distal oesophagus of all subjects but remained >6.0 in the proximal (unexposed) oesophagus. The most common symptom reported with acid infusion was nausea. Other sensations included a cold sensation in the chest region, feeling of hunger and / or heartburn.

3.3.1 Demographic Data

A total of 20 healthy volunteers were recruited and assessed for criteria eligibility. The majority (about 85%) of the subjects who responded to the adverts had a medical background (hospital staff or medical students), the rest were mostly students or research staff from a local university. The age range was from 18-41 years with a mean age of 28 ±5.87years. There were no obese or underweight subjects and the average body mass index (BMI) was 23.59 ±2.43kg/m^2. The subjects were recruited from different ethnic backgrounds reflective of local ethnic diversity. The majority of subjects were Caucasians (76%) followed by Asians (12%), Africans (6%) and Chinese (6%). All were acid infusion naïve, and 88% sensitised to acid infusion. Both sensitisers and non-sensitisers to acid were recruited for study 1.

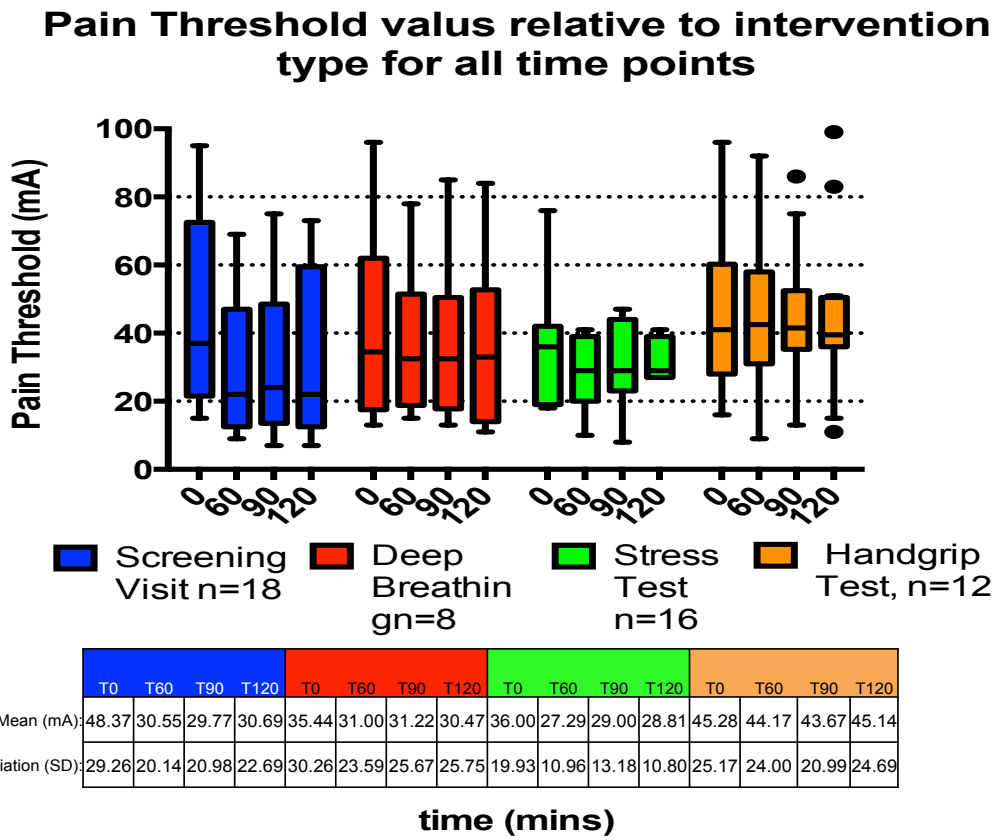
During screening visit two subjects could not tolerate prolonged nasal intubation, even though intubations were successful, and a further subject threw up during acid infusion, contaminating the proximal oesophagus, and had to be excluded. 17 Subjects completed the screening visit protocol and were subsequently randomised into three groups for their second visits by means of a 'pseudo-block-randomisation', as the different modulation types were initially started one at a time, until all three were 'up-and-running' and formal

randomisation could be instigated. One more subject dropped out for their third visit, as he could not attend his final visit due to unexpected travel commitments. For the final analysis, 17 subjects (13 male) were included from the screening visit protocol, 12 subjects from the deep breathing protocol, 12 subjects from the isometric handgrip protocol, and seven subjects from the psychological stress protocol. (Figure 56)

3.4.2 Pain Tolerance Threshold Data for Proximal Oesophagus

The proximal oesophageal pain threshold (PT) data unexpectedly showed that all three modulations caused desensitisation, i.e. no decrease in the difference of mean pain threshold (Δ Avr PT) with regard to the that observed during the screening visit, across all post acid time points. Figure 57(A) & table B below illustrate the average absolute PT values.

A:



B:

	T0	T60	T90	T120	T0	T60	T90	T120	T0	T60	T90	T120	T0	T60	T90	T120
Mean (mA):	48.37	30.55	29.77	30.69	35.44	31.00	31.22	30.47	36.00	27.29	29.00	28.81	45.28	44.17	43.67	45.14
Standard Deviation (SD):	29.26	20.14	20.98	22.69	30.26	23.59	25.67	25.75	19.93	10.96	13.18	10.80	25.17	24.00	20.99	24.69

Table 2

Figure 57 (A&B) Absolute values for proximal oesophageal pain thresholds before (T0) and at T60, T90 and T120, post acid infusion, with (blue) screening visit, (red) deep breathing, (green) stress induction and (orange) isometric exercise. (n-values as stated)

Two-way MANOVA analyses, comparing the screening visit's mean ΔPT for the proximal oesophagus with that of the other modulations, across all time points and with regard to modulation type, showed a statistical difference for deep breathing and isometric exercise. (Figure 58(A)) In comparing average means of pre/post-acid PT differences (Δ Avr PT – '*degree of sensitivity*') between screening visit and modulations; no statistical difference was found for psychological stress modulation, however there was a clear statistical difference seen for both deep breathing and isometric exercise. (Figure 58(B)) There was also no statistical difference in the PT between the mean differences of deep breathing and psychological stress modulation. This suggests that the

psychological stress modulation and isometric exercise did not facilitate an increase in sensitisation to acid during the pilot study.

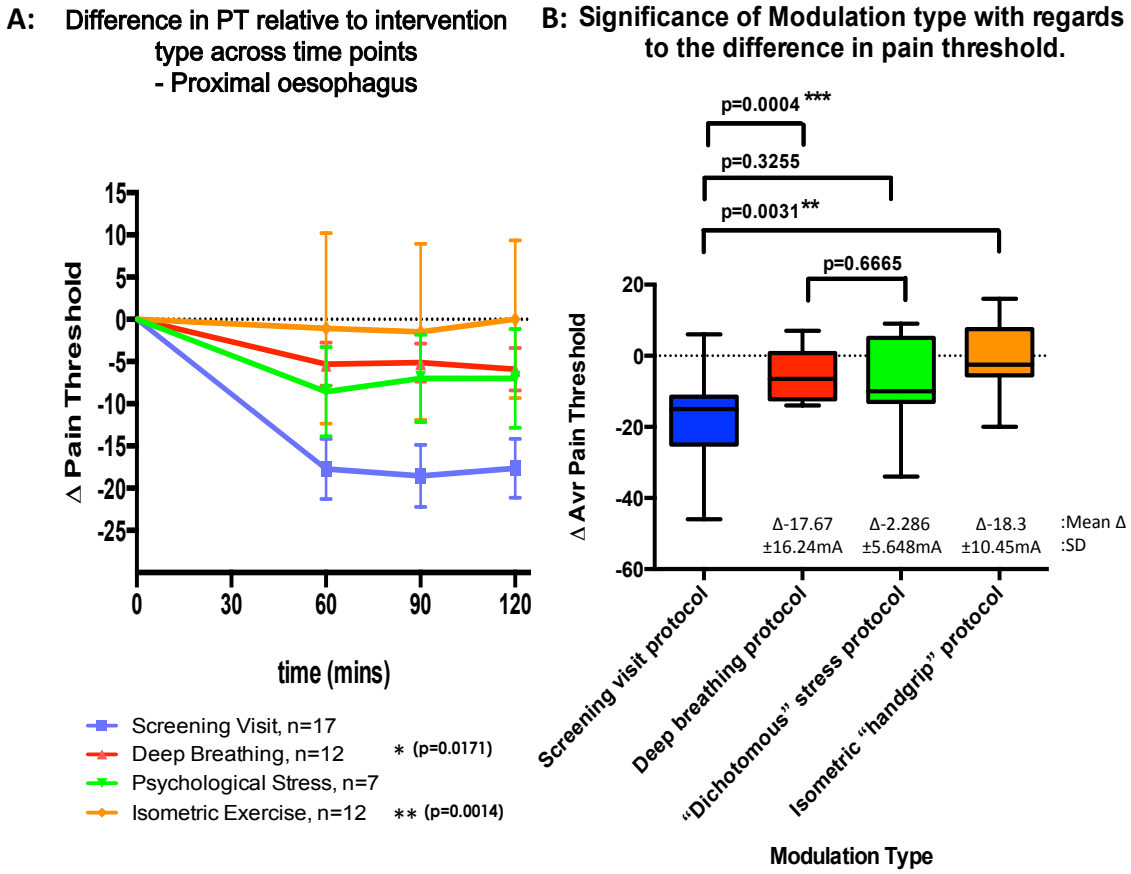


Figure 58 A: ***Shows the difference in mean pain threshold (ΔPT) in mA, for the proximal oesophagus between baseline and the three-time points (minutes) after acid infusion, for the different modulation types.*** **B:** ***Shows the difference in average means of pain threshold (Δ Avr PT) in mA, for the proximal oesophagus between pre & post acid infusion, for the different modulation types.***

3.4.3 Pain Tolerance Threshold Data for Foot

The foot pain threshold data showed that all three modulations caused no significant change with regard to the screening visit. There was thus no indication of any degree of sensitisation with regard to the somatic control (foot) demonstrated in this instance for both MANOVA analyses (Figure 59), and on average PT means comparison of pre/post-acid differences (Δ Avr PT, not illustrated). For all MANOVA analysis data stets were of similar in variance and found to be statistically matching.

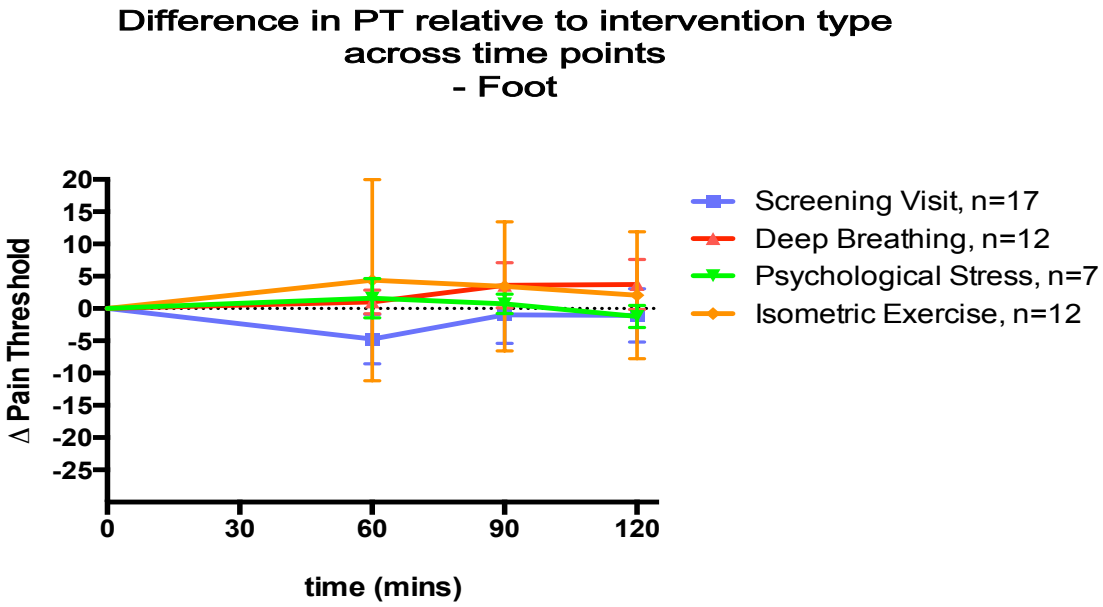
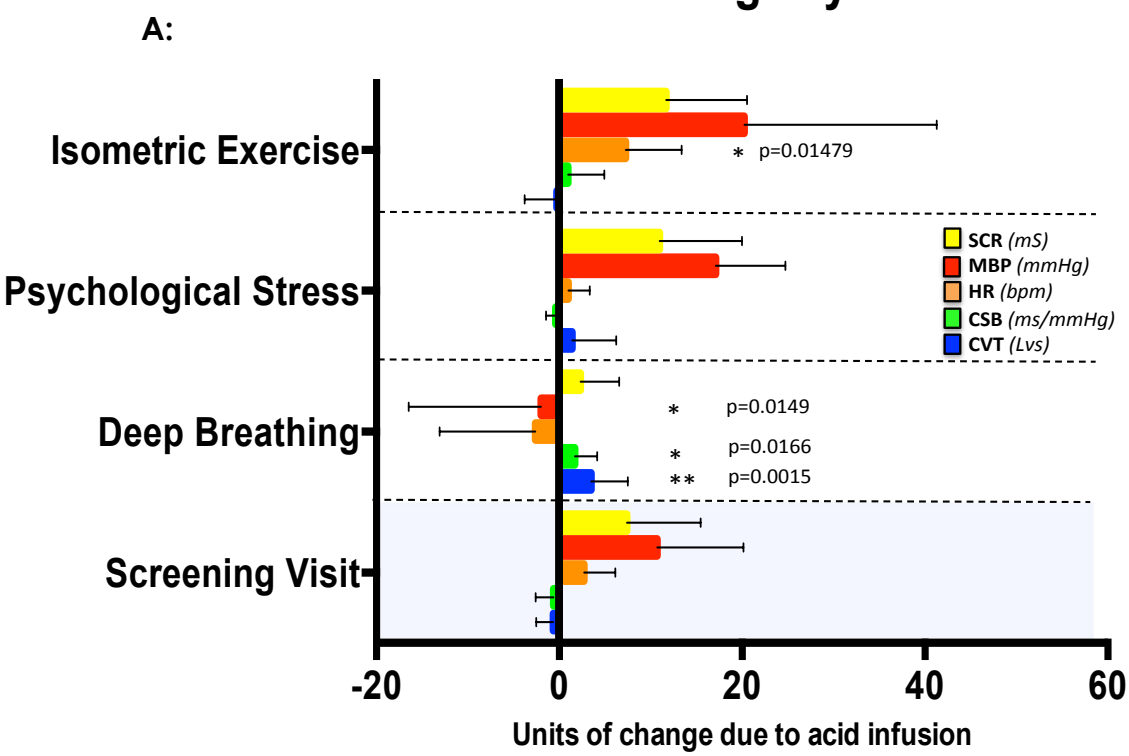


Figure 59 Shows the difference in mean pain threshold (ΔPT) in mA, for the foot between baseline and the three-time points (minutes) after acid infusion, for the different modulation types.

3.4.4 Autonomic Data

The pre/post-acid change in ANS during the screening visits served as the 'baseline' with which other modulation-ANS changes were compared, and is illustrated below. (Figure 60(A) & table 3(B)) The changes observed for screening visit protocol demonstrated (Figure 60(A) - shaded graph), is a post-acid increase in SNS activation (SCR & MBP), with a coinciding PNS withdrawal (CSB & CVT). The SNS is hence 'unopposed' in this instance.

A:



B:

Table 3

Modulation Protocol	ANS Measure	A Avr	SD	Difference between means	P value
Isometric Exercise	SCR (mS)	11.70	8.85	4.308 ± 3.164	0.3476
	MBP (mmHg)	20.25	21.03	9.549 ± 5.804	0.1120
	HR (bpm)	7.28	6.12	4.552 ± 1.796	0.0176
	CSB (ms/mmHg)	0.96	3.95	1.599 ± 1.121	0.1655
	CVT (Lvs)	-0.26	3.51	0.3925 ± 1.015	0.7021
Psychological Stress	SCR (mS)	10.98	9.00	3.591 ± 3.741	0.3476
	MBP (mmHg)	17.13	7.59	6.427 ± 4.290	0.1490
	HR (bpm)	1.00	2.34	-1.729 ± 1.515	0.2664
	CSB (ms/mmHg)	-0.40	1.06	0.252 ± 0.849	0.7696
	CVT (Lvs)	1.42	4.80	2.080 ± 1.351	0.1385
Deep Breathing	SCR (mS)	2.34	4.22	-5.052 ± 2.762	0.0794
	MBP (mmHg)	-1.98	14.50	-12.68 ± 4.819	0.0149
	HR (bpm)	-2.62	10.47	-5.354 ± 2.762	0.1508
	CSB (ms/mmHg)	1.71	2.43	2.335 ± 0.9034	0.0166
	CVT (Lvs)	3.50	3.98	4.160 ± 1.151	0.0015
Screening Visit	SCR (mS)	7.39	8.07		
	MBP (mmHg)	10.71	9.45		
	HR (bpm)	2.73	3.41		
	CSB (ms/mmHg)	-0.64	1.96		
	CVT (Lvs)	-0.65	1.86		

Figure 60 The comparison between the different 'pre/post-acid infusion' ANS changes between screening visit (shaded) and other modulation-types, are graphically illustrated in (A); with table (B) below showing the mean values of change & standard deviations for each specific protocol, and for the three modulations; there respective comparison & p-value significance with regards to the screening visit. [Abbreviations are as follows; SCR: skin conductance response, MBP: mean blood pressure, HR: heart rate, CSB: cardiac sensitivity to baroreflex, & CVT: cardio vagal tone.]

Immediately above (Figure 60(A) - one above shaded graph) is seen the ANS changes as modulated by the deep breathing protocol. There is a distinct and significant difference in activation for this modulation compared to screening and all other visits, as there is a marked activation of the PNS, with a withdrawal of the SNS. The PNS increase is thus uniquely associated with a reduction in SNS activation.

The changes observed during the psychological stress protocol (Figure 60(A) - two above the shaded graph) showed that there was no significant difference in activation for this modulation compared to screening, except for an increase in the magnitude of SNS outflow. Of note is that there was also an increase in CVT, which could be indicative of PNS co-activation, and needs further clarification. Changes for the isometric exercise protocol (Figure 60(A)) demonstrated a similar ANS activation pattern as observed for screening. The main difference being a twofold increase in SNS outflow. Contrary to expectation, there is almost no change with regard to the PNS.

In figure 58(A&B) it is shown that isometric exercise led to a desensitisation with a reduction in Δ Avr PT (post-acid sensitivity) when compared to screening visit, which was an unanticipated result, as the opposite response was intended. However of particular interest here is that this desensitisation is seemingly not associated with an increase in CVT (PNS) outflow, as observed during deep breathing. (Figure 60) To assess this apparent conflicting phenomenon further analysis of the CVT change during the acid infusion period was undertaken.

CVT-data during the 30minute acid-infusion period was analysed in seven data 'collection bins' (each lasting approximately 4.5-minutes).

This was due to a technical limitation of the Neuroscope that only allows for a maximum period of 5 minutes of information to be analysed accurately at any given moment.

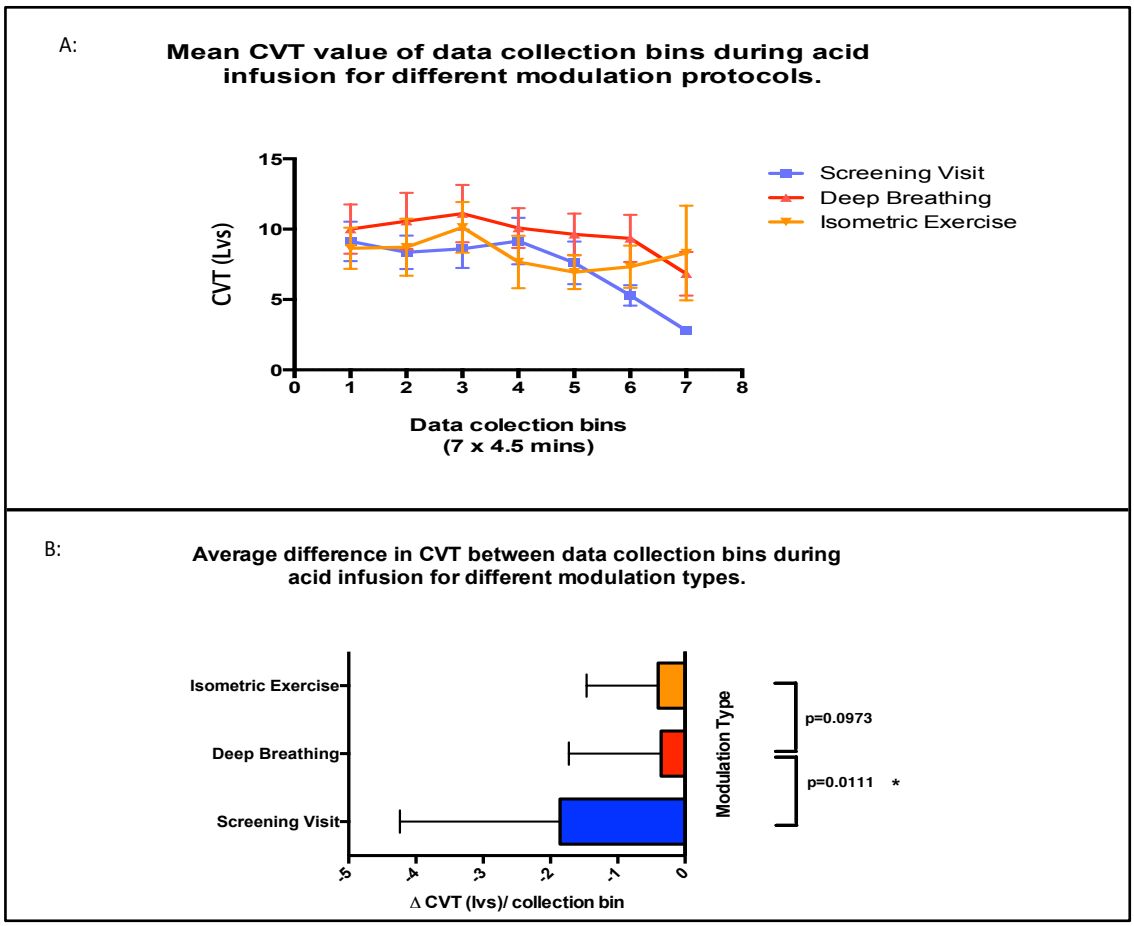


Figure 61 A: ***Graph plotting the change for each individual data collection bin during the 30minute acid infusion period. B: Mann-Whitney test showing the comparison of the average difference between collection bins.***

The average of all seven bins was used for comparative analysis. (Figure 61(A)) When the change for each individual bin is plotted (Figure 61(B)) the graph gradient of isometric exercise (orange) is similar to that of deep breathing (red), in comparison to screening visit (blue). In comparing average differences in CVT per collection bin, (Mann-Whitney) t-test indicated a difference between screening visit and deep breathing, and no difference between deep breathing and isometric exercise. This finding suggests that the net activation of the isometric

exercise modulation is similar to that of the deep breathing for some of the time during acid infusion, and dissimilar in others causing an 'overall-cancellation', and hence a neutral average for the infusion time in total, compared to screening visit, where the withdrawal increases with duration of infusion time.

3.4.5 Psychological Profiling Data

3.4.5.1 BFI questionnaire

On the Big five Inventory (BFI) subjects were scored based on:

1. **Extraversion** – (outgoing/energetic [100] vs. solitary/reserved [0]).
2. **Agreeableness** – (friendly/compassionate [100] vs. cold/unkind [0]).
3. **Conscientiousness** – (efficient/organised [100] vs. easy-going/careless [0]).
4. **Neuroticism** – (sensitive/nervous [100] vs. secure/confident [0]).
5. **Openness to experience** – (inventive/curious [100] vs. consistent/cautious [0]). [10]

There is no maximum value subjects could achieve using the BFI questionnaire. Therefore the percentage of maximum possible (Cumulative Percentages) was used to interpret the data. Cumulative Percentages is a linear transformation of raw metric data, which is graded into a 0 to 100-percentile scale. Where 0 represents the minimum possible score and 100 represents the maximum possible score on the continuum between the opposites of the specific personality trait. Cumulative Percentage scores is a universal metric that is more intuitive than scale scores with idiosyncratic ranges. (265)(Figure 62)

[10] For a more detailed description see explanatory note, appendix Three.

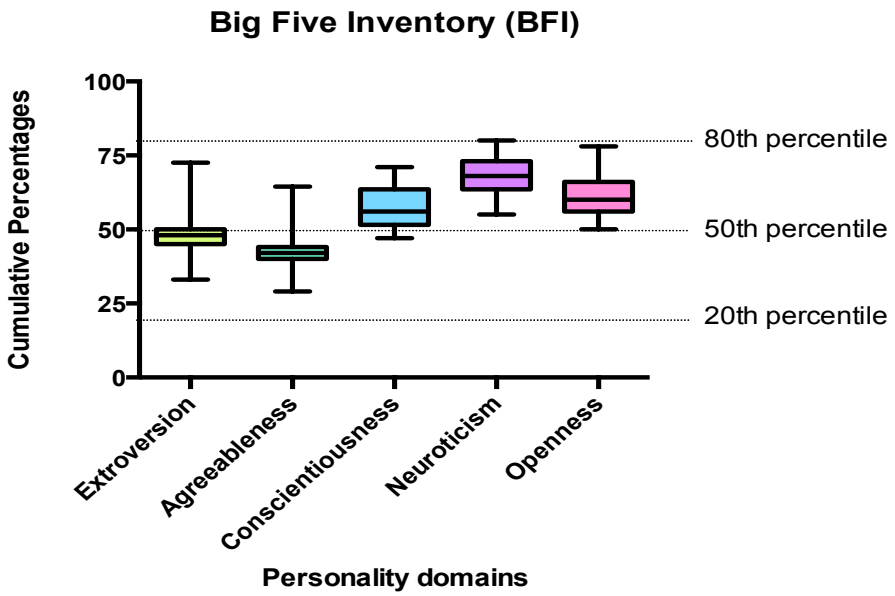


Figure 62 ***Big Five inventory (BFI) as percentage of maximum possible (Cumulative Percentages) scores in n=17 healthy volunteers across the five different personality domains.***

The personality domains of this cohort of healthy volunteers indicated that they were evenly grouped between introversion and extroversion. Their agreeableness was just below the 50th percentile, whereas their conscientiousness, neuroticism and openness were above the 50th, but below the 80th percentiles. There were no personality extremes detected (above the 80th, or below the 20th percentile – indicative of personality disorders). Neuroticism, a personality vulnerability factor, was the highest, with openness, a protective personality factor, the second highest. Based on the BFI average response the cohort as a whole could be described as a "semi-social, slightly reserved, organised, emotionally sensitive but curious and adventurous" group. This personality description would be expected from a cohort where the majority of individuals are in or are training to be in the caring professions (conscientious with emotional sensitivity (neuroticism)). It is further suggestive and consistent of the self-selection that occurs with advert recruitment, as only the individuals with a high degree of openness (curiosity and

adventurousness) will willingly volunteer for invasive experimentation as required by this study.

3.4.5.2 Hospital Anxiety and Depression questionnaire

On the Hospital Anxiety and Depression Score (HADS) the mean values for anxiety, 9.18 ±2.51(SD) and depression, 8.41 ±1.28(SD) were within the borderline range (HADS score of 8-10/21), but below the clinical 'caseness' cut-off (HADS score of ≥11/21)(266), but 29% of subjects met the criteria for moderate anxiety and 6% for moderate depression. This is reflective of the efficacy of exclusion criteria used during recruitment, as none of the subjects attracted a formal psychiatric diagnosis and as such were not on any psychotropic medication. These individuals are examples of the more extreme end of a healthy cohort, with a high percentage being university students and are of interest as they are potentially representative of a large part of the patient group clinically seen. (200) Hence the cohort was slightly anxious, but still representative and consistent with expected means for age and gender of the general population. [8]

Analysis of the State and Trait Anxiety Inventory (STAI) indicated firstly that the cohort's trait anxiety, 38.53 ±7.22(SD) is consistent with general population expectations (38.69 ±10.34(SD)). (267) A second finding was that the subjects' state anxiety reduced with each subsequent visit (mean ΔSTAI-S = -2.395 per visit), and is an example of exposure habituation. With regard to emotional attachment style, only 13% of subjects had significant attachment vulnerability as measured by the Vulnerable Attachment Style Questionnaire (VASQ), and due to the small sample size most probably represent a type II error.

3.4.6 Correlation Data

During Screening visit a statistically significant positive correlation between the differences in PT and SCR was detected, r=0.505 (p=0.039). This implies that visceral sensitivity increases with the increase in sympathetic outflow. (Figure 63)

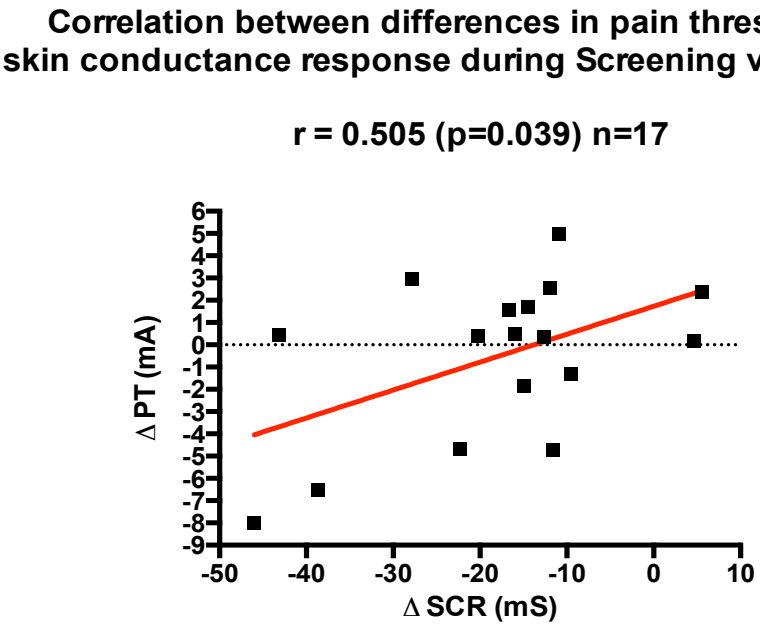


Figure 63 ***The correlation between the difference in pain threshold (ΔPT) and skin conductance response (ΔSCR) during screening visit.***

Also seen during the screening visit was that the difference in SCR (ΔSCR) correlated positively with HADS-anxiety, r=0.491 (p=0.045), WAI-distress, r=0.528 (p=0.029) and TAS, r=0.4596 (p=0.012).[11] This implies that an increase in sympathetic outflow correlates with anxiety, distress and the inability to read or understand subject's subjective emotional state. For

[11] Bonferroni correction was not used for these observations.

this study there was no significant correlation found with neuroticism for any of the visits. A strong negative correlation between the differences in PT and CVT was found during the Deep breathing protocol, r=-0.753 (p=0.031). (Figure 64) Also seen was a strong negative correlation between the differences in PT and CSB, r=-0.817 (p=0.013), implying that an increase in both afferent and efferent branches of the para-sympathetic nerves system is associated with a reduction in the degree of visceral sensitivity, and replicates previous findings using this model. (30)

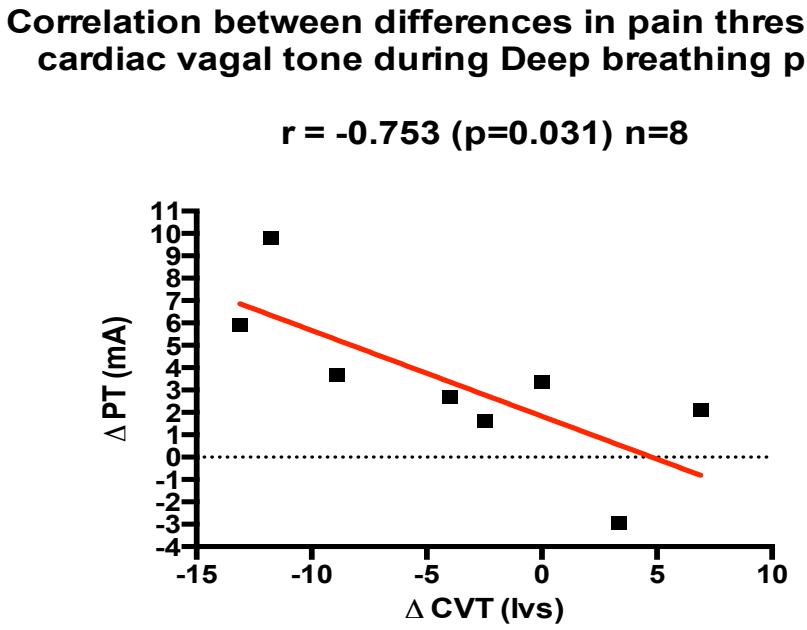


Figure *64 The correlation between the difference in pain threshold (ΔPT) and cardiac vagal tone (ΔCVT) during deep breathing visit.*

In comparing both the sympathetic and para-sympathetic activation, a divergent effect regarding their respective correlations with the average PT is observed during Deep breathing. Sympathetic activation as measured by the difference in SCR has a strong negative correlation, r= -0.766 (p=0.016), while para-sympathetic as measured by the differences in CVT, r= 0.787 (p=0.02) have strong positive correlations. Thus, the higher

the average PT, the higher the para-sympathetic activation and inversely, the bigger the degree of the sympathetic withdrawal.

3.5 Summery of Key findings for study 1 (Modulation Pilot Study)

3.5.1 Demographic Data:

1. 85% of the subjects had a medical background.
2. All subjects were acid infusion naïve, and 88% sensitised.
3. The pilot study was at 60% power.

3.5.1 Pain Tolerance Thresholds Data:

1. The distal oesophageal pain threshold data showed all three modulations caused desensitisation, with regard to the Screening visit, across all time points.
2. The foot pain threshold data showed all three modulations caused no significant change with regard to the Screening visit, across all time points.
3. Deep breathing desensitised significantly at, -18.3 ±10.45mA p=0.0004, with p=0.0171 across all time points.
4. Isometric exercise desensitised significantly at, -17.67 ±16.24mA, p=0.0031 with p=0.0014 across all time points.
5. Psychological stress modulation did not significantly desensitise at, -2.286 ±5.648mA p=0.3255, with p=0.1187 across all time points.
6. Non-sensitisers remained desensitised for all modulations.
7. Non-sensitisers were markedly less vulnerable to the effects of stress induction p=0.0201.

3.5.2 Autonomic Data:

1. Screening visits demonstrated a post-acid increase in sympathetic outflow, with a para-sympathetic withdrawal. (SNS increase unopposed by PNS)
2. Deep breathing protocol demonstrated a post-acid/modulation decrease in sympathetic outflow, with a statistically significant increase in para-sympathetic activation, CSB: 2.33 ±0.90ms/mmHg p=0.017, and CVT: 4.16 ±1.15Lvs p=0.002. (PNS increase with no SNS co-activation)
3. During Psychological stress modulation there was no objective indication of ANS stress, with no significant difference in ANS regulation post-acid/modulation demonstrated. (SNS was increased with PNS co-activation)
4. Isometric exercise protocol showed a difference in an increase in the magnitude of SNS outflow, with a statistical increase for HR, 4.55 ±1.80bpm p=0.018. (Twofold increase in SNS, with 'neutral' PNS)
5. Isometric exercise protocol, during the 30minute acid infusion period, showed that the degree of change per data collection bin was more similar to that of the Deep breathing, than to that of the Screening visit, and no statistical difference was detected between Deep breathing and Isometric exercise during data bin analysis, p=0.097.

3.5.3 Psychological Questionnaire Data & Correlations:

1. The lower the volunteers' pain threshold (Avr PT, p=0.049), or the higher their degree of sensitivity (ΔPT, p=0.016); the more likely the subjects were to report the stimulus as more painful or unpleasant.

2. Neuroticism, a personality vulnerability factor was the highest, with openness, a protective factor the second highest. (BFI)
3. The cohort's mean personality profile indicated a "semi-social, slightly reserved, organised, emotionally sensitive but curious and adventurous" study group. (BFI)
4. 29% of subjects met the criteria for moderate anxiety and 6% for moderate depression. (HADS)
5. Subjects' state anxiety reduced with each subsequent visit with a mean difference in score of 2.4 per visit. (STAI-S)
6. Significant positive correlation between ΔPT and ΔSCR was detected, r=0.505 (p=0.039), during Screening visit.
7. A strong negative correlation between the differences in ΔPT and ΔSCR was found during the Deep breathing protocol, r=-0.753 (p=0.031).
8. The higher the average PT, the higher the para-sympathetic activation and inversely, the larger the degree of the sympathetic withdrawal, SCR, r=-0.766 (p=0.016), CVT, r= 0.787 (p=0.02) and CSB, r=0.717 (p=0.045).
9. The higher the emotional valence, the lower the pain threshold, HADS anxiety, r=-0.900 (p=0.006), and depression, r=-0.809 (p=0.028).
10. There were no significant correlations found with neuroticism for any of the visits.

3.6 Discussion

This study replicates and further demonstrates the finding that oesophageal acidification in a validated model of human oesophageal pain hypersensitivity is associated with sympathetic nervous system activation, and parasympathetic withdrawal. (30) The data presented

further suggest that activity within the ANS during oesophageal acidification correlates with the degree of subsequent sensitisation. Using this model, previous studies have shown that the magnitude of sensitisation is variable between individuals with some failing to sensitise. (175, 191) The factors responsible for variability in sensitisation in the model were unknown; this study examined how this variability could be influenced by psychophysiological factors, and the preliminary data suggests that autonomic nervous system's activity is associated with variability in sensitisation to acid infusion.

The data presented showed that the initial hypothesis was confirmed stating that sensitisation as expressed by the difference in pain threshold (ΔPT) was directly proportional to SNS activation (ΔSCR), and PNS withdrawal (ΔCVT). Objectively, for the first time it was demonstrated that psychophysiological induced unopposed PNS activation successfully produced a subsequent statistically significant desensitisation in the acidified oesophagus. Subjectively, the reporting of pain and discomfort was found to be proportional to the degree of hypersensitivity, and it was observed that subjects' pain and discomfort during acidification was also reduced during positive PNS modulation.[12] However, the SNS could not be further amplified by stress or exercise induction. Pain thresholds on the foot did not change significantly from baseline with acid infusion suggesting that hypervigilance was not the mechanism of the increased pain sensitivity in the oesophagus.

This study also replicates, and independently verifies the previous finding that anxiety is a significant vulnerability factor, and that it increases the magnitude of acid-induced oesophageal pain hypersensitivity in healthy

[12] For more information see appendix 4.1.

volunteers. (179, 211, 268) Objective evidence is thus further provided to support the hypothesis that anxiety influences the degree of post-injury pain hypersensitivity in the human oesophagus. The data presented also highlighted the role and relationship between affective states (anxiety, depression and distress), ANS activation and the subsequent degree of visceral hypersensitivity. Even though this study failed to demonstrate a further increase in the degree of sensitisation by means of stress induction, it still confirmed that stress plays a role in the degree of visceral sensitisation.

Supporting this, it has been reported clinically that increased comorbid anxiety scores significantly predict the diminished symptomatic response to PPI therapy in both endoscopy positive and negative cases. (269) This data suggests that anxiety both promotes the development of sensory dysfunction and hinders the resolution of aberrant sensory processes in response to therapy once dysfunction is established. A recent study by Rubenstein *et al.* (270) demonstrated that oesophageal sensation in patients with heartburn was correlated with the presence and degree of psychological dysfunction. Compared to healthy volunteers, patients with heartburn demonstrating VPH had lower sensory and pain thresholds to oesophageal balloon distension. However, when these patients underwent oesophageal acid perfusion, the presence of psychiatric factors such as anxiety was associated with increased pain intensity and discomfort suggesting that anxiety modulates visceral sensory processing.

Regarding the psychological stress induction modulation; the data presented indicated that the use of dichotomous listening, a previously validated method to examine the impact of stress on visceral perception in IBS patients, (246-248) was unsuccessful in further increasing the

degree of hypersensitivity in the healthy volunteers above that which was already established during screening visit. Contrary to the initial hypothesis the modulation protocol produced a degree of desensitisation in the proximal oesophagus compared to the secondary hyperalgesia observed at screening visit. This observation is most likely due to the study not being at full power and producing a type II error, the changes seen were not of statistical significance, but remain important. ANS regulatory markers indicated an increase in SNS and PNS outflow (increased SCR, MBP & CVT) that is suggestive and consistent with of autonomic co-activation.

Paine *et al.* (271) found that the pain induced by balloon distension in the proximal oesophagus of healthy volunteers, evoked "fight-n-flight" responses with novel parasympathetic/sympathetic co-activation, and that the personality traits correlated with the slope of distal oesophageal pain-related CVT changes, where the more neurotic-introvert subjects had greater sensitivity. The data presented indicated that the cohort investigated in the current study measured highest for neuroticism on the BFI, and hence these findings may have a bearing on the observed ANS response. Porges *et al.* (70, 272) with the polyvagal theory demonstrated that in greater stress situations the more primitive vagal nucleus (dorsal motor nucleus - DMNX) contrary to expectation increases activation to produce greater PNS outflow to cause co-activation with the SNS. The branches of the vagus nerve serve different evolutionary stress responses in mammals: the more primitive branch (DMNX) elicits immobilisation behaviours (e.g., feigning death), whereas the more evolved branch from the nucleus Ambiguous is linked to social communication and self-soothing behaviours. (See section 1.9.4, chapter 1, page 53) These functions follow a phylogenetic hierarchy, where the most primitive systems are activated only when the more evolved structures fail. The

experimental measurement of vagal tone has since become a novel index of stress vulnerability and reactivity and could be an objective indication of the stress induction protocol's effect on SNS and PNS co-activation which precluded any effect of SNS modulation on pain sensitivity. (273)

The data presented showed no significant correlations found with neuroticism for any of the visits, even though it was a prominent personality factor in the cohort. Drabant *et al.* (274) reported that neuroticism mediated autonomic and neural responses during threat anticipation, where the intensity varyed as a function of the threat anticipated, and Coen *et al.* (275, 276) demonstrated that higher neuroticism is associated with engagement of brain regions responsible for emotional and cognitive appraisal during anticipation of pain but reduced activity in these regions during the actual experience of the pain. It could hence be that the main influence of the degree of neuroticism is not effectivly evaluated in the response to an isolated pain incident, as simulated by this model, but that it would be more reflective in situations of prolonged anticipation of pain.

There is further the confounding factor of self-selection to consider that occurs with advert recruitment, as only the individuals with a high degree of openness (curiosity and adventurousness) will volunteer for invasive experimentation. Firstly the high degree of openness might not be representative of the clinical population, and secondly it is a protective factor when exposed to stress induction, and might influence results obtained. Another confounder is the fact that a high percentage of the subjects had a medical or research background, and hence are familiar with the laboratory environment or procedures. The reduced novelty has a negative impact on the anticipation, as is represented by the low baseline STAI anxiety scores measured. This then enhances the exposure

habituation observed as reflected by the subjects' state anxiety reducing with each subsequent visit, and affects the degree of visceral sensitivity to repeated stimuli. Labus *et al.* (277) demonstrated normalisation of visceral hypersensitivity following repeated exposure to experimental visceral stimuli in IBS patients. (277)demonstrating that they experienced decreased discomfort and pain thresholds to visceral stimuli, including cognitive change in hypervigilance to gastrointestinal sensations, and to the context in which these visceral sensations and symptoms occurred. In addition Sharma, using this model demonstrated that the magnitude of acid-induced sensitisation is variable between visits and that sensitisers may show diminishing sensitisation with repeated studies. (30)

A final important factor to consider regarding the psychological stress induction modulation specifically, but applying equally to all modulation protocols used, is that previous studies showed that distraction reduces the degree of visceral pain sensitivity. (276, 278) In 1968 Melzack and Casey (279) described pain in terms of its three "dimensions":

I. Sensory- discriminative (location, intensity, quality, duration),
II. Motivational- affective (emotional valence, suffering and urge to escape the suffering), and
III. Cognitive- evaluative (contextual meaning and degree of attention/ focus).

At the Screening visit the subject has no distraction during the period of acid infusion, and is fully focused on the visceral sensation, while during all subsequent visits during the same period they are in addition also engaged with one of the modulation protocols. In the case of stress induction, it could produce a reduction, while during the deep breathing protocol the effect could be additive.

In a similar study, Fass *et al.* used the dichotomous listening stress paradigm to assess the effect of distress on the perception of intraoesophageal acid in patients with gastro-oesophageal reflux disease, and found that it was effective in producing hypersensitivety only in patients, and could not reproduce visceral sensitisation in healthy controls. (280) This suggests that in the absence of a predisposing vulnerability the stress intensity produced by this paradigm is not of a magnitude to produce the intended effect required in healthy volunteers, and can explain the lack of sensitisation observed in our study. To overcome similar problems while assessing the effect of stress in Inflammatory Bowel Disease patients, Goodhand *et al.* (249) amended the protocol to include a standardised reading and mental arithmetic task to perform while listening to the dichotomous auditory recording. The inclusion of this amendment, along with the minimisation of acid exposure visits, and the addition of an active placebo to control for the effect of distraction, should be incorporated in future modulation studies.

Concerning the use of the Isometric handgrip exercise protocol as positive modulator of the sympathetic nervous system, the data presented was unexpected, as the anticipated response was that of an increase in sympathetic tone, associated with the withdrawal in parasympathetic tone. Previous studies used this method successfully to increase sympathetic tone. (162, 244, 245) Here however the stimulation paradigm was for a far shorter time duration and was effective as a positive SNS modulator. For my study however the isometric handgrip exercise was used over a 30-minute period during acid infusion. Each handgrip exercise period lasted for five minutes, and was repeated at least three times during the infusion period.

On *post hoc* ANS data analyses it became clear the when the isometric handgrip exercise was used repeatedly over a longer time period it exhibited different physiological properties. Closer laboratory observation revealed that towards the end of the 5-minute exercise period, as the muscle excursion becomes more evident, subjects, as part of the 'straining to maintain the grip', would unconsciously hold their breath and effectively perform a prolonged Valsalva manoeuvre. When this manoeuvre is analysed using the Neuroscope, it becomes clearer that the Valsalva manoeuvre produces a short initial SNS increase and PNS withdrawal, but is then followed by a large PNS rebound increase, caused by the increased outflow from midbrain regulatory centres in response to the positive thoracic pressure stimulation of baro- and-chemoreceptor situated in the carotid sinus and aorta arch. (Figure 65)

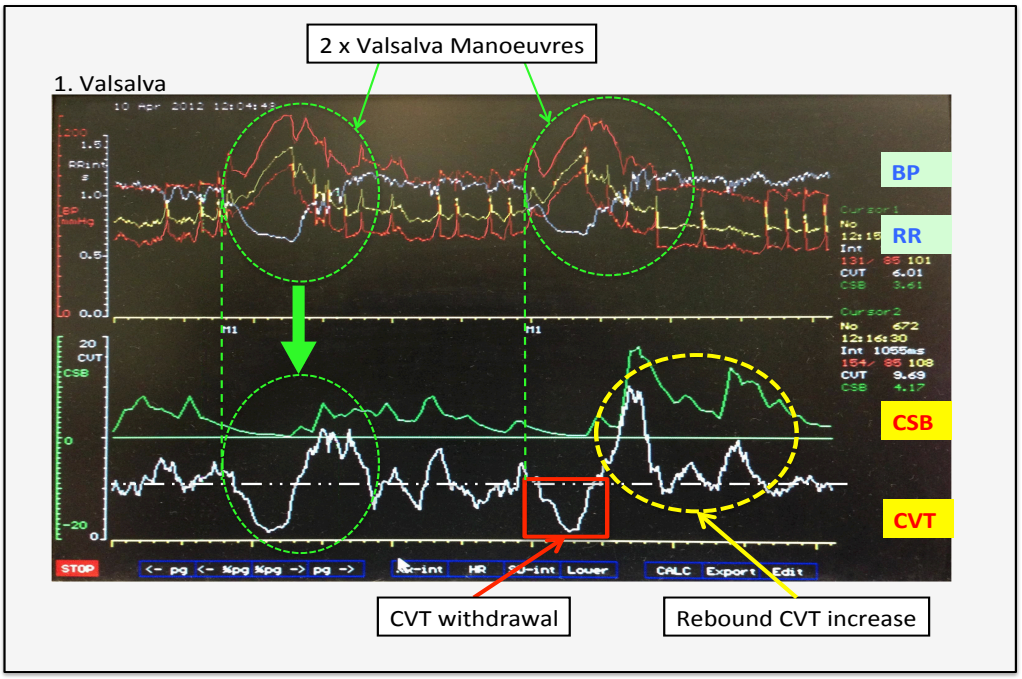


Figure 65 ***This diagram shows the Neuroscope 'screenshot' analysis of a subject performing two Valsalva manoeuvres. The red box highlights the coinciding decrease in CVT from baseline followed by a large rebound increase in PNS activity highlighted by the yellow oval. The graphs in the upper half of each panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of etch panel shows the CSB (green graph) and CVT (white graph) each labelled as such.***

Neuroscope analysis of the isometric handgrip exercise revealed similar physiological responses to that observed during the Valsalva manoeuvre. (Figure 66) It thus clarifies why the resultant CVT change measured during the isometric handgrip protocol was neutral (-0.26 ±3.51Lvs, Figure 60), as the produced CVT withdrawal is effectively cancelled by the rebound PNS increase. Analysis of the data collection bins during the acid infusion period confirms this, as the net CVT change during the handgrip protocol was not significantly different from that which was observed during the deep breathing protocol, and would explain the significant subsequent oesophageal desensitisation observed. (Figure 61) The repeated use of the isometric handgrip exercise protocol over a longer time period inadvertently turned out to be similar to the deep breathing protocol at producing desensitisation in this model.

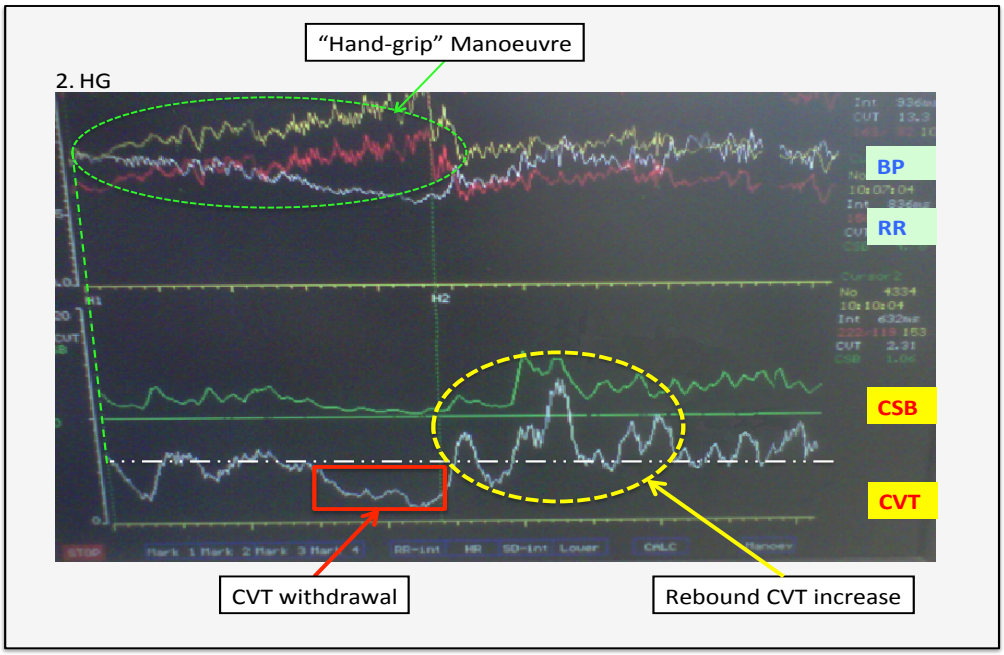


Figure 66 This diagram shows the Neuroscope 'screenshot' analysis of a subject performing he Isometric handgrip exercise. The red box highlights the coinciding decrease in CVT from baseline followed by a large rebound increase in PNS activity highlighted by the yellow oval. The graphs in the upper half of each panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of etch panel shows the CSB (green graph) and CVT (white graph) each labelled as such.

The only feasible alternative to producing prolonged SNS increase associated with PNS withdrawal is the use of the tilt-table test (TTT), (281) which can be effective over time periods as long as 20 to 30 minutes. Its costly, cumbersome equipment is associated with frequent dizziness and syncope, making its possible use for this study impractical given the present circumstances. Future investigators will have to find a more appropriate alternative physiological method to examine the effect of sympathetic increase on sensitisation in this model.

Concerning the use of the Deep breathing protocol as positive modulator of the para-sympathetic nervous system, it would suffice to say at this stage that data presented was novel and encouraging, and will be fully discussed in the following chapter. The deep breathing modulation protocol successfully demonstrated the proof of concept that increasing parasympathetic outflow can produce visceral desensitisation in this model. Due to the desensitising effect of distraction (as discussed for the "dichotomous listening" protocol), Deep breathing as intervention should be tested against an active placebo. This should now be investigated further with a fully powered study.

Finally, in spite of all subjects being acid infusion naïve, 22% did not sensitise during acid infusion, and also failed to sensitise on subsequent visits, irrespective of modulation. They also demonstrated that acid-induced autonomic responses are variable between visits. For these subjects the difference in degree of sensitisation between visits was not related to the degree of change in HR, CVT or CSB between visits. They had less sympathetic activation (SCR) compared to sensitisers, and scored less for neuroticism on the BFI. Their response remained consistent, and remains unclear and is suggestive that they may represent a distinct

phenotype with reduced susceptibility to injury-induced sensitisation in the model. As a previous study using this model demonstrated that stress induction increases the degree of secondary oesophageal hyperalgesia in sensitisers, (30) it now remains to further investigate this in non-sensitisers.

3.7 Conclusions

As study 1 was a pilot study and not yet fully powered the emphasis was on identifying early trends to investigate more thoroughly in the subsequent studies, hence the following conclusions were made:

1. The Isometric "handgrip" exercise test is not suitable for this investigation, as it produces both a parasympathetic withdrawal and rebound increase. The alternative "tilt-table" test is impractical in the circumstances, and future investigators will have to find a more appropriate method to examine the effect of sympathetic increase on sensitisation in this model.
2. Due to subjects' anxiety habituation as measured by the STAI, the decreased induction of acid induced sensitisation on subsequent visits and the "first-pass effect" of the screening visit, the amount of visits should be kept to a minimum. The screening visit should be discontinued, and randomisation should occur directly following recruitment. Recruitment should attempt to include a larger diversity of backgrounds to reduce subjects familiar with medical environment.
3. As previous studies have already demonstrated that stress increases the degree of sensitisation in sensitisers using this model, the augmented Psychological stress protocol should be performed only

in non-sensitisers, as the effect on non-sensitisers has not been studied and remains unknown.

4. The Deep breathing modulation successfully demonstrated the proof of concept that increasing parasympathetic outflow can produce visceral desensitisation in this model. Due to the desensitising effect of distraction (as discussed for the "dichotomous listening" protocol), deep breathing as intervention should be tested against an active placebo. This should be investigated further with a fully powered study.
5. The inter-individual variability in the magnitude of sensitisation between sensitisers and non-sensitisers, as well as their vulnerability and protective factors remains unclear and as yet unexamined, and should now be further explored in a comparative study, that will allow the evaluation of ANS responses across a continuum of environmental stress for the different sensitisation groups.

4 Effect of Psychophysiological Modulation by Deep Breathing & Psychological Stress on Acid Induced Oesophageal Hypersensitivity

4.1 Introduction

Visceral pain in FGID is a major global cause of disability, healthcare seeking and a leading cause for loss of quality of life and patient morbidity. (23) Chronic visceral pain is a common condition for which patients seek care from various health-care providers. This type of pain causes much suffering and disability, but in spite of its ubiquity is still misunderstood and undertreated and tangible patient benefit remains limited. (282) Visceral pain shares many features with somatic pain, yet there are important differences in the pathophysiological mechanisms underlying visceral nociception, but these differences are not reflected in treatment approaches to date. (283) Few controlled clinical trials of psycho-behavioural interventions for pain relief in FGID exist in spite of frequent support for their importance as adjuncts to medical treatment. (284)

Ashburn *et al.* (285) in a landmark discussion of the long-term care of patients suffering from chronic pain conditions, state that these patients often require adjustment of treatment with the aim of decreasing pain and suffering while improving physical and mental functioning, and they cite behavioural interventions including training in deep breathing amongst effective interventions in the management of this difficult to treat patient group. Similarly Syrjala *et al.* (286) researching in cancer related visceral pain, found that training of deep breathing, relaxation and guided imagery, reduced cancer treatment-related pain to such a

degree, that adding cognitive-behavioural skills to the breathing-relaxation regimen did not further improve pain relief. Observations of this kind have led to the use of deep breathing in pain control becoming more widespread. Using data from the 2002 National Health Interview Survey (NHIS), conducted by the Centres for Disease Control and Prevention National Centre for Health Statistics, it was found that in 2002 already 12% of patients in the USA used deep breathing exercise strategy in the treatment of chronic pain. (287) Deep breathing exercises now form a standard part of the non-pharmacological treatment for patients with chronic widespread pain and fibromyalgia. (288)

With regard to pain control in the acute A&E setting, Downey *et al.* (289) found that the usefulness of deep breathing exercises was ineffective in reducing acute pain levels statistically; however noted that the majority of patients who received deep breathing education felt it was useful. The exercise was effective in increasing patients' feelings of rapport and motivation to follow their doctors' directives, both of which are key features in the effective management and treatment of chronic pain.

Finally concerning the clinical use of deep breathing, Jerath et al. (290) described a specific variation of deep breathing (long pranayamic[13] breathing) in the treatment of autonomic nervous system and other related disorders. They described a physiological response characterised by the presence of decreased oxygen consumption, decreased heart rate, and decreased blood pressure, as well as increased theta wave amplitude in EEG recordings, with increased parasympathetic activity, accompanied by the psychological experience of alertness and reinvigoration. The exact mechanism of how deep breathing interacted

[13] *Prana (प्राण, prāṇa) is the Sanskrit word for breath or "life force".*

with the nervous system affecting metabolism and autonomic nervous system changes remains to be clearly understood. This model however validated the hitherto poorly understood modulation effects of deep breathing as a topic requiring more research, especially in context of chronic visceral pain management.

Similar autonomic findings to those described were observed during study 1 described in chapter three. Using the deep breathing protocol, it was demonstrated that increasing parasympathetic outflow could produce oesophageal pain desensitisation in a model of acid induced hypersensitivity. Study 1 was, however, a pilot study and not fully powered with the emphasis being on identifying early trends. Further investigation of this desensitising effect of deep breathing and the underlining mechanisms was therefore undertaken. Due to the desensitising effect of distraction as discussed in chapter three, deep breathing as intervention was now tested against an active placebo using a blinded crossover study design at full power.

Also discussed in chapter three was the use of stress induction, and the role of the stress responses in oesophageal sensitisation. It is in this regard that Paine et al. (271) demonstrated that oesophageal intubation evoked "fight-flight" responses with heart rate and sympathetic (CSI, SC, MBP) activation and significant parasympathetic (CVT) withdrawal ($p<0.05$). Sharma *et al.* (185) went on to demonstrate that the degree of resulting oesophageal sensitisation to acid correlated with the degree of vagal withdrawal. Porges proposed vagal tone as a novel index of stress vulnerability and reactivity with potential applications in all branches of medicine. He further proposed a model emphasising the role of the parasympathetic nervous system and particularly the vagus nerve in mediating homeostasis and defining the degree of stress. (273)

Hausken *et al.* (291) investigated the effects of acute mental stress on gastric antral motility in 23 healthy volunteers and 25 patients with functional dyspepsia. They found that the sympathetic tone increased during stress in both groups. Vagal tone was however lower in the functional dyspepsia patient group than in the healthy controls ($p < 0.001$). The lack of stress-related reduction of motility among patients with functional dyspepsia may, therefore, be a consequence of poor vagal tone, supporting Porges's hypothesis where vagal tone acts as a novel index of stress vulnerability.

Mayer *et al.* described that different types of stress play an important role in the onset and modulation of irritable bowel syndrome (IBS) symptoms. They demonstrated the physiological effects of psychological and physical stressors on gut function and brain-gut interactions, and highlighted that they are mediated by outputs of the emotional motor system in terms of autonomic, neuroendocrine, attentional, and pain modulatory responses. (110) Posserud *et al.* confirmed this by demonstrating that stress worsens IBS symptoms. They hypothesised that the stress effect might be explained by altered neuroendocrine and visceral sensory responses to stress in IBS patients, as they found that stress induced exaggeration of the neuroendocrine response and visceral perceptual alterations during and after the exposure to stress. This may explain some of the stress related gastrointestinal symptoms in IBS patients. (292)

In study 1 the examination of the effect of stress was attempted in context of the acid induced oesophageal hypersensitivity model. Unfortunately the psychological "dichotomous listening" stress test was found to be ineffective most likely due to a lack in stress intensity, and

therefore requires augmentation as suggested by Goodhand *et al.* (249) The protocol was hence amended to also include a standardised reading and mental arithmetic task, to be completed during the "dichotomous listening" stress test.

Study 1 demonstrated that in spite of all subjects being acid infusion naïve, 22% did not sensitise during acid infusion, and also failed to sensitise on subsequent visits, irrespective of modulation. For these subjects the difference in degree of sensitisation between visits was not related to the degree of change in HR, CVT or CSB between visits. They had less sympathetic activation (SCR) compared to sensitisers, and scored less for neuroticism on the BFI. It is possible that they may represent a distinct phenotype with reduced susceptibility to injury-induced sensitisation in this model. A previous study using this model demonstrated that stress induction increases the degree of secondary oesophageal hyperalgesia in sensitisers, (30) but the response in non-sensitisers remains still unclear. It was decided to focus further investigation in this area.

In study 1 it was noted that due to subjects' anxiety habituation, there was a reduced induction of acid induced sensitisation on subsequent visits. Thus the amount of visits should be kept to a minimum. The screening visit was hence discontinued, and randomisation occurred directly following recruitment. Recruitment also attempted to include a larger diversity of volunteer backgrounds to reduce subjects familiar with the medical environment. The stress augmentation together with the minimised number of acid exposure visits, and the addition of an active placebo to control for the effect of distraction and personal interaction, was now used to examine the effects of stress induction in non-sensitising individuals during study 3.

The aim of this study was thus to determine the effects of psychophysiological modulation of the ANS by means of deep breathing (study 2), and psychological stress induction (study 3), on acid-induced oesophageal pain. Also to ascertain if inter-individual differences in the degree of sensitisation were predicted by inter-individual differences in psychological profile. In order to best achieve this aim, the study was divided into two parts, with study 2 focusing on the effect of PNS increase (SNS withdrawal) in individuals that sensitise to acid, and study 3 focusing on the effects of PNS withdrawal (SNS increase) in individuals that do not sensitise to acid. It was hypothesised that sensitisation as expressed by the difference in average pain threshold (ΔPT) would be directly proportional to sympathetic nervous system activation (SNS: ΔSCR), and parasympathetic nervous system withdrawal (PNS: ΔCVT), as induced or amplified by different psychophysiological modulations. A secondary aim of the study was to expand on the data in order to determine whether psychological state and trait factors predicted the degree of sensitisation to acid in the model.

4.2 Materials and Methods

4.2.1 Ethics Committee Approval

All protocols for this study were submitted and approved by the University Senate Ethics Committee, 'East London and The City Research Ethics Committee - Alpha' (ref: 09/H0704/71). See section 2.1(page 77).

4.2.1 Subjects

55 healthy asymptomatic adult male and female volunteers, aged 18 to 50, were recruited by advertisement. In an attempt to include a larger

diversity of backgrounds to reduce subjects familiar with the medical environment, adverts were placed in waiting areas open to the public. Adverts were also placed at a local university's main campus where non-medical students attend. Pre-screening for inclusion and exclusion criteria was completed as described in section 2.2 (page 77).

4.2.2 Location of the LOS

As in study 1 we had determined that the LOS position could be accurately identified using the pH pull through technique therefore this technique (as described in section 2.3 page 78), was used for the subjects in studies 2 & 3.

4.2.3 Other Methods of Measurement

All other methods of measurement; Catheter Assembly (section 2.4, page 78), Oesophageal acid infusion (section 2.4, page 78), Oesophageal pH monitoring (section 2.6, page 80), Pain Threshold Measurements (section 2.8, page 82), Psychological assessment (section 2.11, page 85), Measurement of the Autonomic Nervous System (section 2.12, page 86) and Respiratory Monitoring (section 2.16, page 99), was performed as described in their specific sections.

4.2.2 Methods of Psychophysiological Modulation

For study 2 & 3 the Sham breathing protocol (section 2.20.2, page 111) and Deep breathing protocol (section 2.20.3, page 114), was used as described in the specific sections, and illustrated in figure 4.1 below. During the Sham breathing protocol subjects were divided into two groups depending on the degree of sensitisation. If they sensitised, they completed study 2 (hypothesis testing study); if they did not sensitise,

they were diverted to study 3 (hypothesis generating study), where the psychological stress induction was achieved by using the augmented "Dichotomous listening" psychological stress test protocol as described in section 2.20.6 (page 119). During study 3 the "standardised reading and mental arithmetic task" was included. (249)

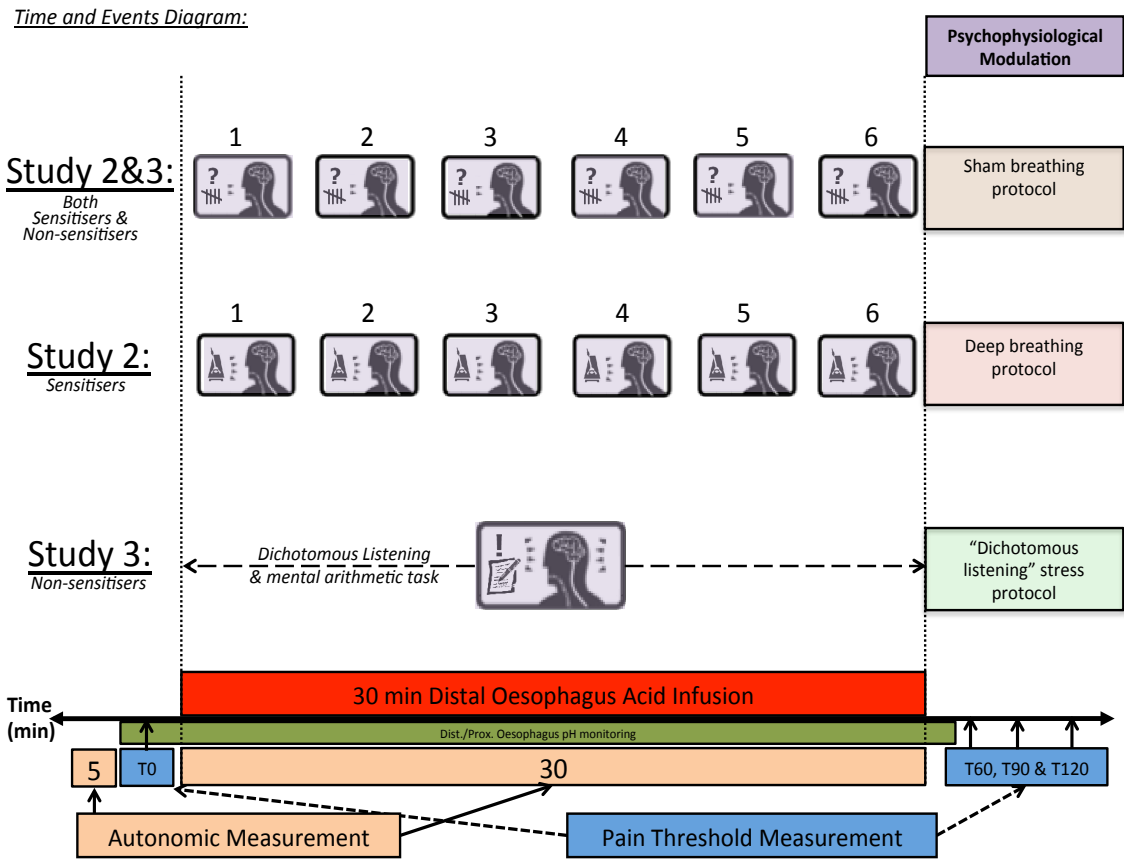


Figure 67 Diagram illustrating the psychophysiological modulation protocol for (from top to bottom) the 'Sham breathing' visit: The subject was asked to count 6 breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar). 'Deep breathing' visit: The subject was paced to perform 6 deep breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar). 'Stress test' visit: Subjects listened to a conflicting duel track sound recording (purple figures) during the 30minitus acid infusion period (red bar). During study 3, it was augmented to include a mental arithmetic task as well. Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).

4.2.3 Study Procedure, Experimental Design & Protocol

The experimental study design was that of a prospective randomised placebo controlled two-tiered crossover double-blinded longitudinal cohort study. (Figure 67) The study procedure was followed as described in section 2.17 (page 99), i.e. using the 'three research assistants' method. The experimental protocol was used as described in section 2.18 (page 101), with 'time and events' proceeding as outlined in figure 2.12 (page 86). Specific modulation protocols were followed as discussed above in section 4.2.2.

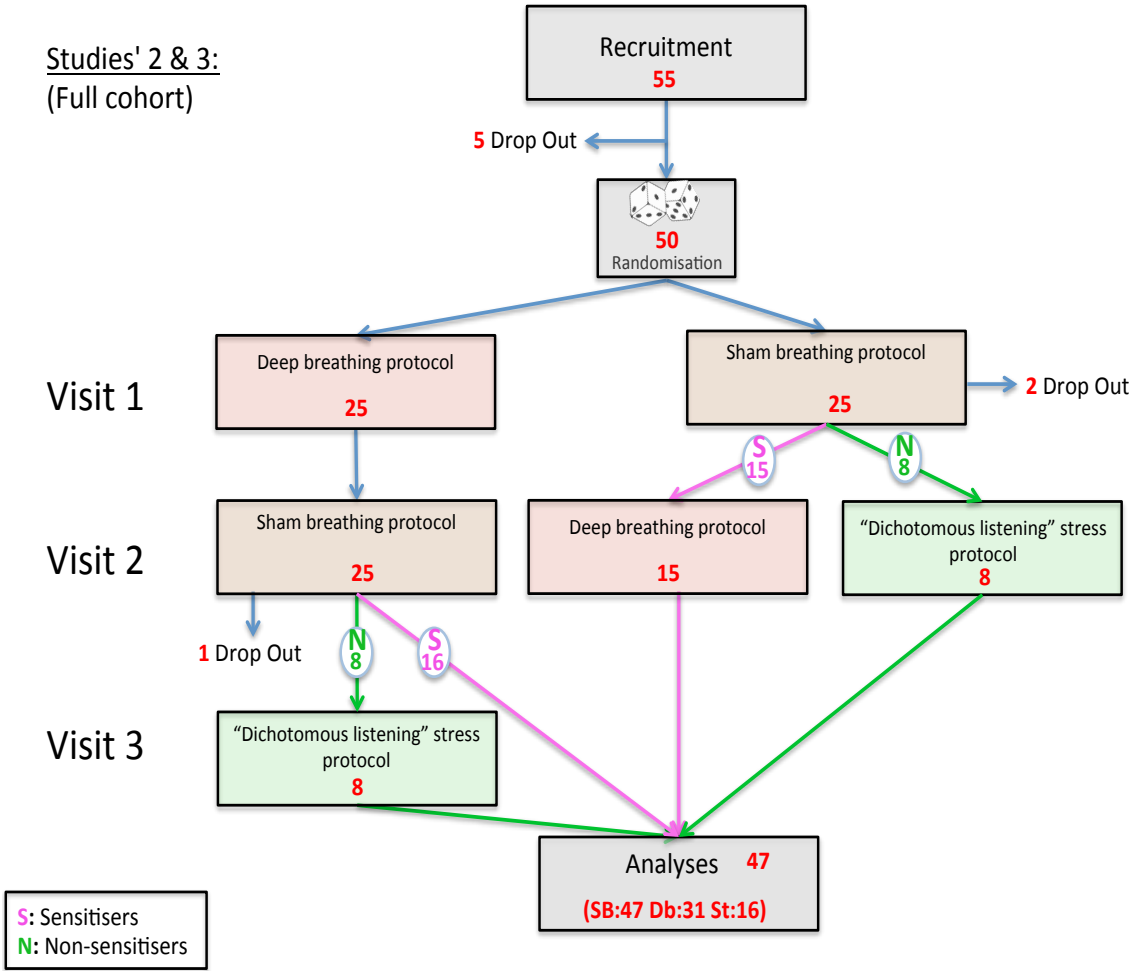


Figure 68 *Flow diagram illustrating the final numeric outcome of participants in studies' 2 & 3. The experimental study design was that of a prospective randomised placebo controlled two-tiered crossover double-blinded longitudinal cohort study. [SB: Sham Breathing, ST: Stress Test, Db: Deep Breathing.]*

4.2.4 Data Handling and Analysis

Due to the methodological dissimilarities (differences in baseline protocol), data derived from study 1 was not pooled or used in the analysis of studies 2 or 3. Demographic, pain threshold and autonomic data were normally distributed hence data are presented as mean ± SD, with parametric analysis. The variability was computed for the main effects of each subject's change in PT over time points (ΔPT & time). All statistical analysis was completed as described in section 2.21 (page 120).

4.3 Results

During acid infusion, pH fell to <2.0 in the distal oesophagus of all subjects but remained >6.0 in the proximal (unexposed) oesophagus. The most common symptom reported with acid infusion was nausea. Other sensations included a cold sensation in the chest region, feeling of hunger and / or heartburn.

4.3.1 Demographic Data for full cohort (studies 2 & 3)

A total of 55 healthy volunteers (31, male) were recruited and assessed for criteria eligibility. In spite of changing our advertising strategy, the majority (about 60%) of the subjects who responded to the adverts still had a medical background (hospital staff or medical students), the rest were other students from a local university and general public. The age range was from 18-48 years with a mean age of 26 ±6.61years. There were no obese or underweight subjects with a mean body mass index (BMI) of 22.73 ±2.41kg/m^2. The subjects were recruited from different ethnic backgrounds reflective of local ethnic diversity. The majority of subjects were Caucasians (63%) followed by Asians (29%) and Africans (4%). All were acid infusion naïve, and 63% sensitised to acid infusion.

Author's note:

To avoid confusion, parts of the results section will be presented separately in the following order:

- Sections 4.3.2 to 4.3.7: *will deal with study 2, (sensitisers) and*
- Sections 4.3.8 to 4.4.11: *will focus on study 3 (non-sensitisers).*

Further results will once again pertain to the whole cohort.

4.3.2 Demographic Data for study 2 (Deep Breathing in Sensitisers)

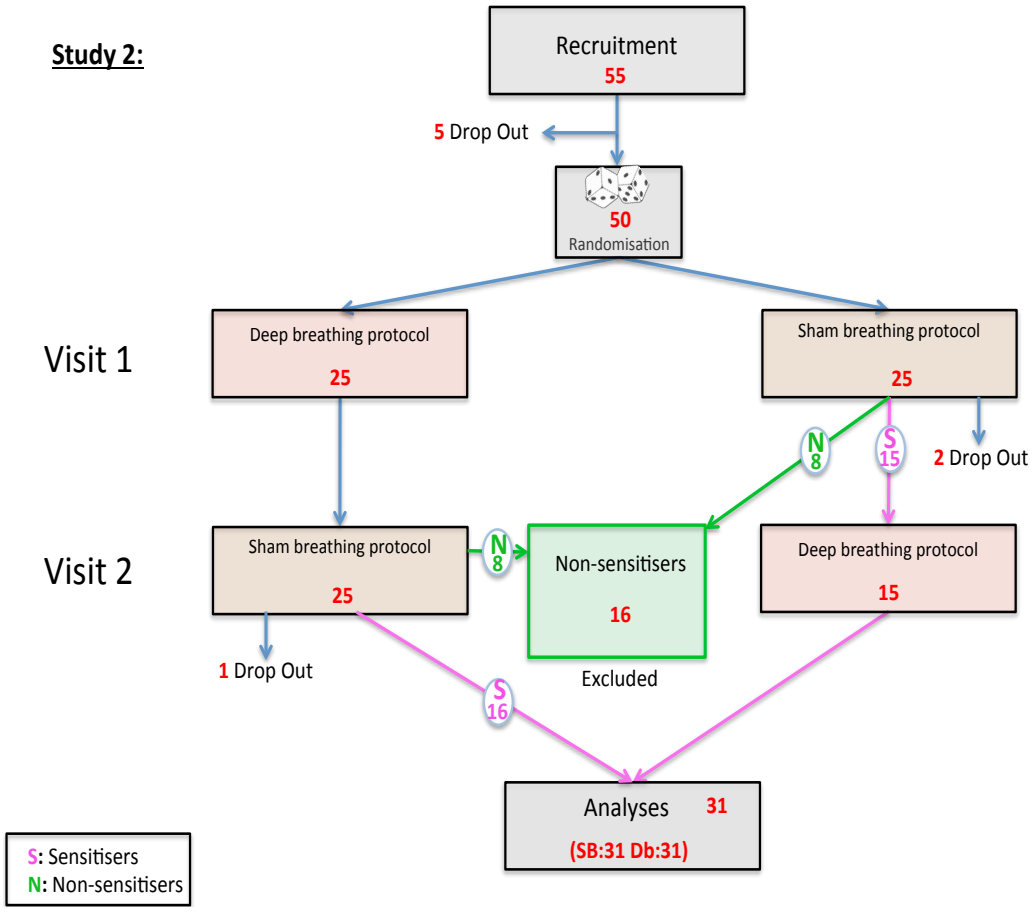


Figure 69 Flow diagram illustrating the final numeric outcome of participants in study 2. The experimental study design was that of a prospective randomised placebo controlled two-tiered crossover double-blinded longitudinal cohort study. [SB: Sham Breathing, Db: Deep Breathing.]

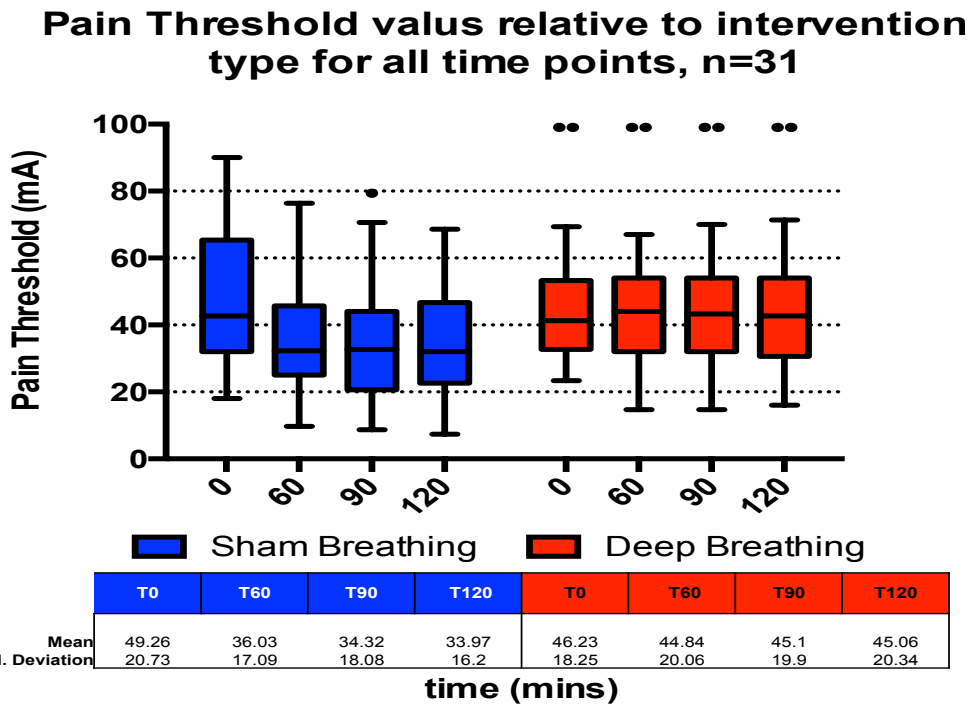
Form the full cohort of 55 healthy volunteers, 31 subjects (18, male) sensitised to acid infusion during the Sham breathing protocol visit, and were recruited to study 2. (Figure 69) The age range was from 18-48 years with a mean age of 26 ±6.28 years. There were no obese or underweight subjects and the average body mass index (BMI) was 22.87 ±2.66kg/m^2. The subjects were recruited from different ethnic backgrounds reflective of local ethnic diversity. The majority of subjects were Caucasians (55%) followed by Asians (35%) and Africans (10%). All were acid infusion naïve.

During their first randomisation visit, five subjects could not tolerate prolonged nasal intubation even though intubations were successful and were excluded. For the final analysis, 31 subjects (18 male) completed both the sham breathing and deep breathing protocols, as a further three subjects were unable to complete the study due to study unrelated reasons (one had a family emergency and the other two sudden unscheduled commitments). (Figure 69)

4.3.3 Pain Tolerance Threshold Data of Proximal Oesophagus for study 2

Absolute threshold data for the proximal oesophagus at (T0) and after acid infusions (T60, T90, T120) are shown in Figure 70(A) & table B below.

A:



B:

	T0	T60	T90	T120	T0	T60	T90	T120
Mean	49.26	36.03	34.32	33.97	46.23	44.84	45.1	45.06
Std. Deviation	20.73	17.09	18.08	16.2	18.25	20.06	19.9	20.34

Table 4

Figure 70 ***Absolute values for proximal oesophageal pain thresholds before (T0) and after (T60 T90 and T120) post-acid infusion with (red) sham and (blue) deep breathing.***

The mean individual 'pre/post-acid infusion' changes in pain threshold (ΔPT) for all subjects in the proximal oesophageal, during sham-&-deep breathing with the mean group value (SD) for each time point, are shown in figure 71.

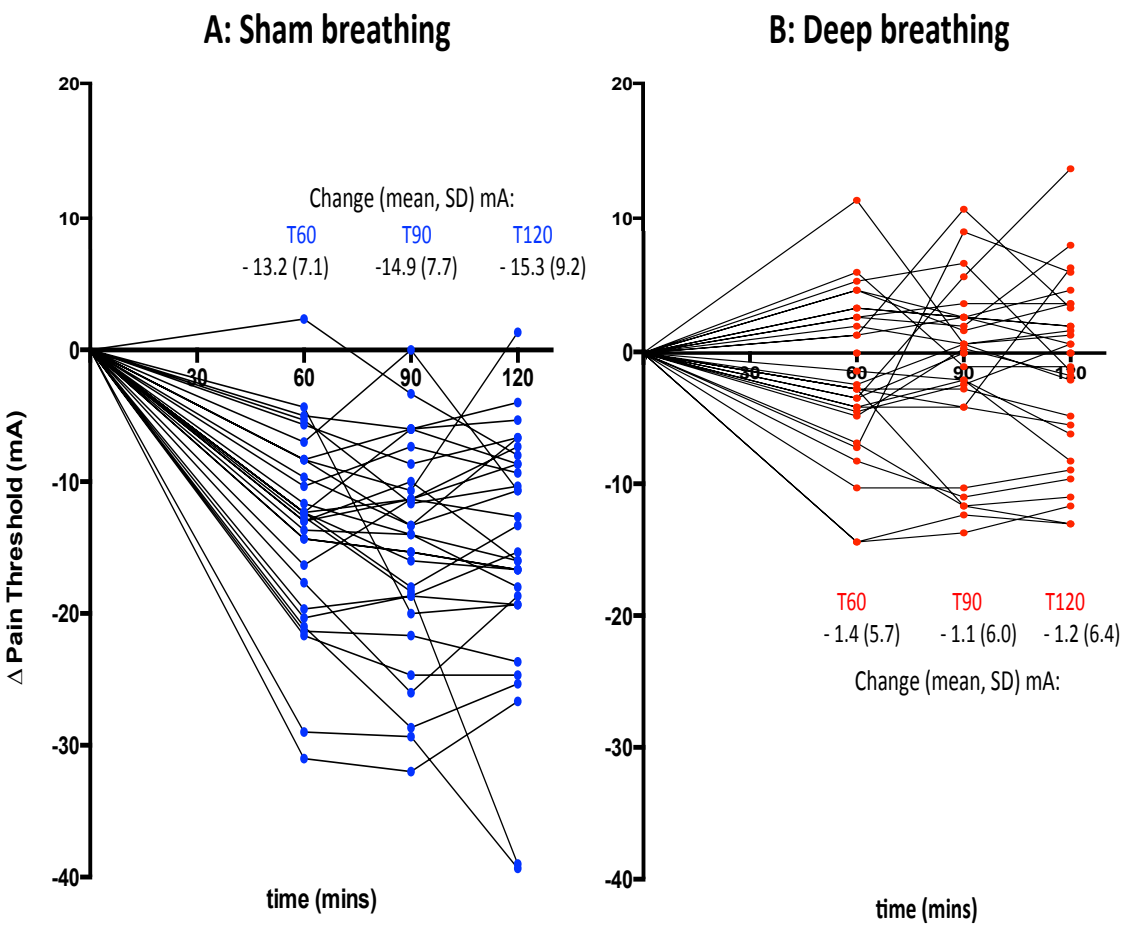


Figure 71 ***Individual values of change (difference from baseline) for proximal oesophageal pain thresholds (ΔPT) for time points T60, T90 and T120, before and after acid infusion with (A) sham and (B) deep breathing.***

Two-way MANOVA analyses, comparing the influence of effect for sham vs. deep breathing modulation's mean ΔPT for the proximal oesophagus with that of modulation type, across all time points, showed a strong statistical significance with regard to deep breathing, contributing 29.85% ($p<0.0001$). (Figure 72(A)) In the comparison of the pre/post-acid infusion differences in average means of pain threshold (Δ Avr PT) between sham breathing, a strong statistical difference was detected between the modulation types. (Figure 72(B)) Deep breathing almost abolished the development of acid-induced hypersensitivity in the proximal oesophagus. At 60 minutes, mean change in PT (C.I.) was -13.2mA (-15.8 to -10.6) after sham breathing, compared to a very small

decrease of -1.4mA (-3.5 to +0.7) after deep breathing. This pattern was repeated at 90 and 120 minutes. Mixed effects regression showed a coefficient of effect for deep breathing of + 9.94 (CI 8.3-11.6), p= 0.0001.

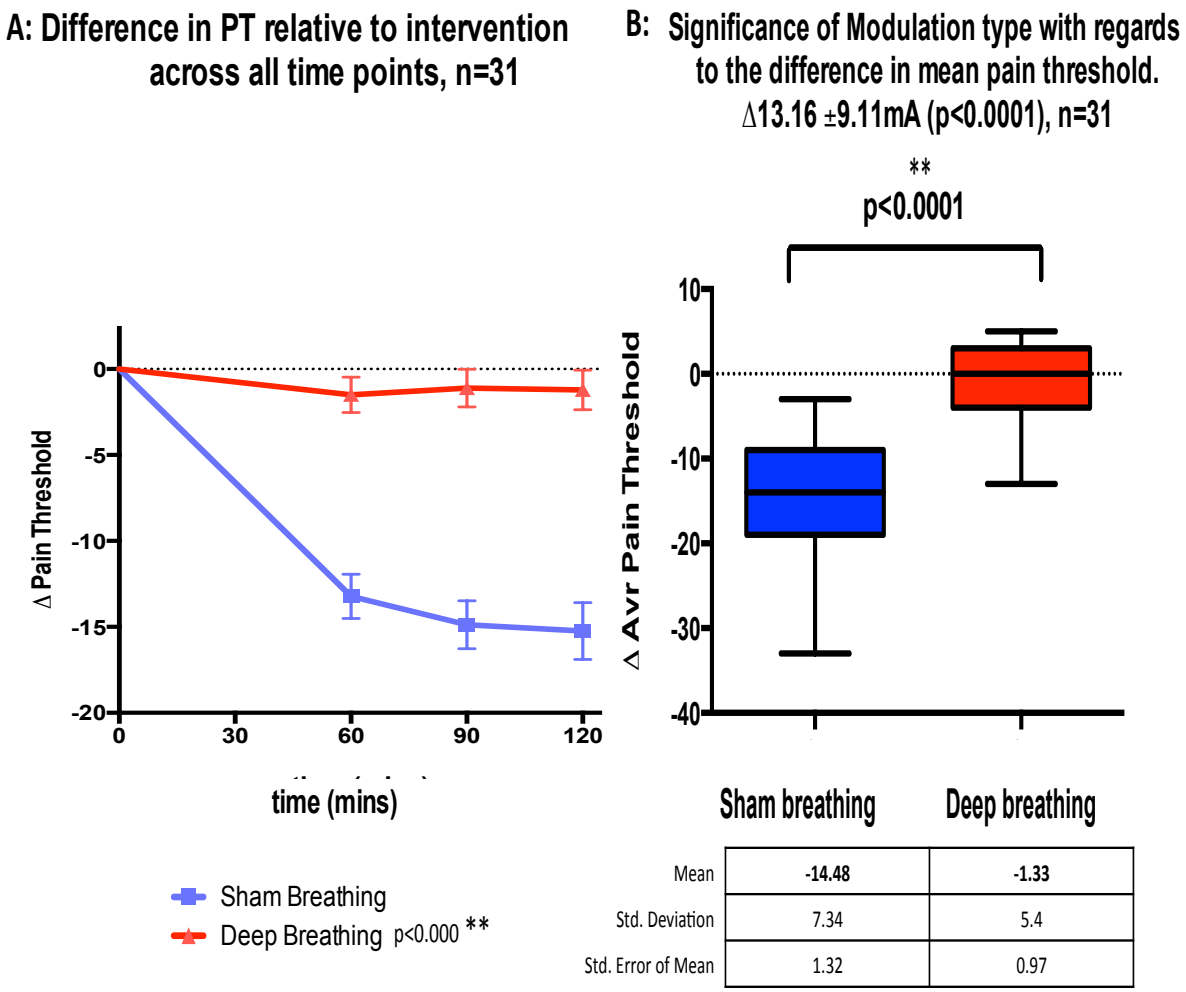


Figure 72 A: shows the difference in mean pain threshold (ΔPT) in mA, for the proximal oesophagus between baseline and the three-time points (minutes) after acid infusion, for the different modulation types. B: Showing the difference in average means of pain threshold (Δ Avr PT) in mA, for the proximal oesophagus between pre & post acid infusion, for the different modulation types. The two-tailed paired t test was statistically significant.

4.3.4 Pain Tolerance Threshold Data of Foot for study 2

The foot pain threshold data showed the deep breathing modulations caused no significant change with regards to sham breathing. There was

thus no indication of any degree of sensitisation with regard to the somatic control (foot) demonstrated in this instance for both MANOVA analyses (Figure 73), and on average PT means comparison of pre/post-acid differences (Δ Avr PT, not illustrated). For all MANOVA analysis data sets were of similar in variance and found to be statistically matching.

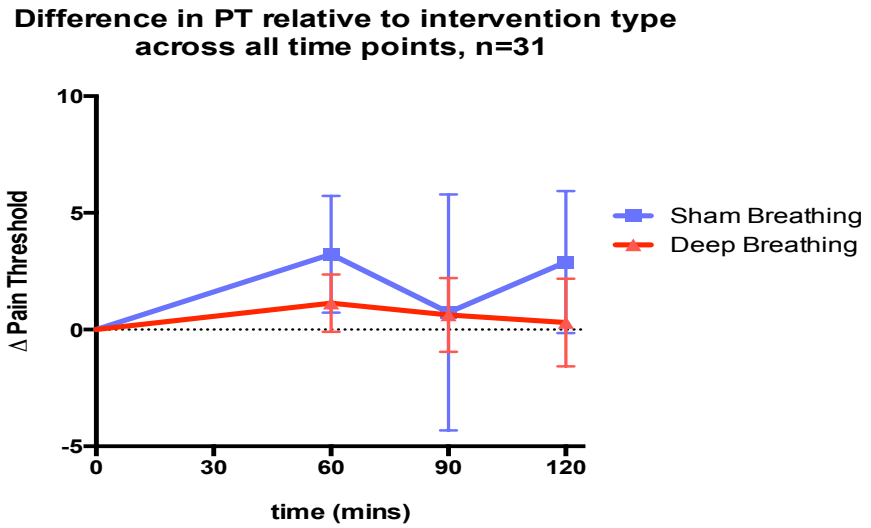
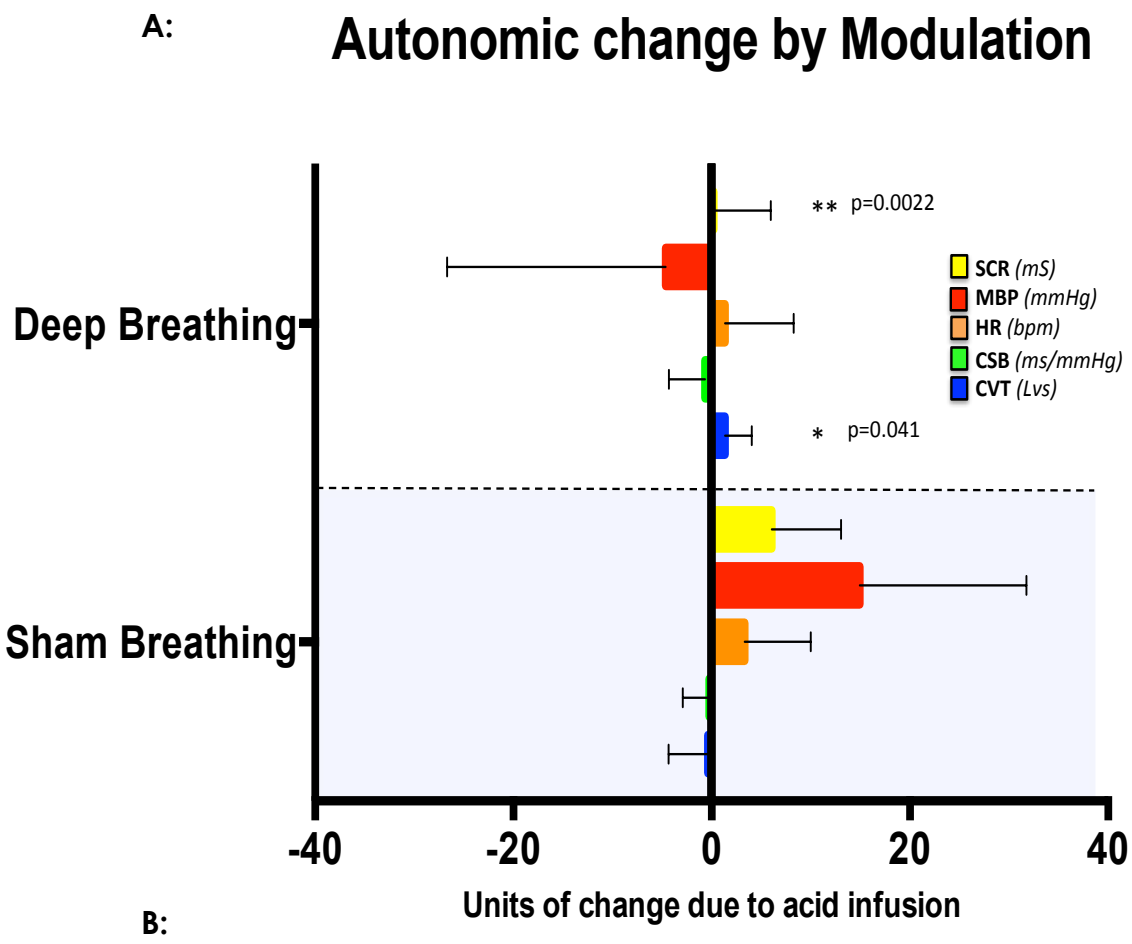


Figure 73 ***Shows the difference in mean pain threshold (ΔPT) in mA, for the foot between baseline and the three-time points (minutes) after acid infusion, for the different modulation types.***

4.3.5 Autonomic Data for study 2

The 'pre/during-acid' infusion change in ANS for sham breathing served as 'baseline' to which deep breathing's ANS changes were compared for p-value calculation, and is illustrated below. (Figure 74(A) & table 5(B))

A:



B:

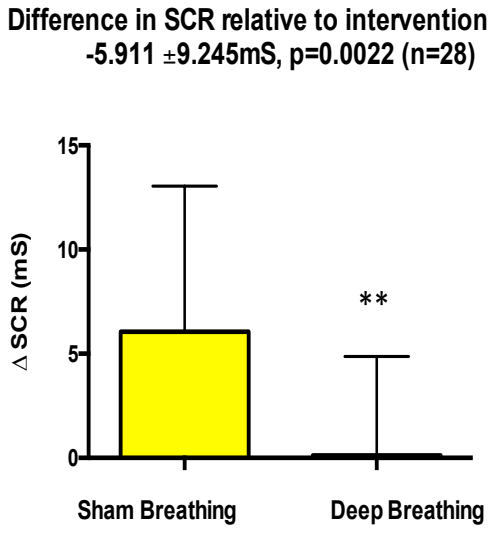
Table 5

Modulation Protocol	ANS Measure	Δ Avr	SD	Difference between means	P value
Deep Breathing	SCR (mS)	0.11	4.75	-5.91 ± 9.24	0.0022
	MBP (mmHg)	5.06	28.19	-9.0 ± 32.51	0.1447
	HR (bpm)	2.07	7.12	-1.13 ± 9.17	0.5168
	CSB (ms/mmHg)	-0.26	3.13	0.24 ± 3.81	0.7351
	CVT (Lvs)	1.7	2.6	2.07 ± 5.12	0.041
Sham Breathing	SCR (mS)	8.73	9.09		
	MBP (mmHg)	15.71	14.02		
	HR (bpm)	2.47	4.78		
	CSB (ms/mmHg)	0.29	2.45		
	CVT (Lvs)	0.53	2.83		

Figure 74 The comparison between the different 'pre/post-acid infusion' ANS changes between sham breathing (shaded) and deep breathing modulation-types, are graphically illustrated in (A); with table (B) below showing the mean values of change & standard deviations for each specific protocol, and for deep breathing's comparison & p-value significance with regards to sham breathing. [Abbreviations are as follows; SCR: skin conductance response, MBP: mean blood pressure, HR: heart rate, CSB: cardiac sensitivity to baroreflex, & CVT: cardio vagal tone.

Sham breathing (shaded graph above, figure 74(A) & table (B)) demonstrated a post-acid increase in SNS activation, with a coinciding PNS withdrawal. The SNS is hence homeostatically 'unopposed' as a result of acid infusion. In contrast the deep breathing protocol showed the reverse, demonstrating a decrease in sympathetic outflow with an increase in parasympathetic activation, with statistically significant changes in SCR & CVT. (Figure 75(A&B)) Modulated deep breathing's PNS increase is thus not associated with SNS co-activation.

A:



B:

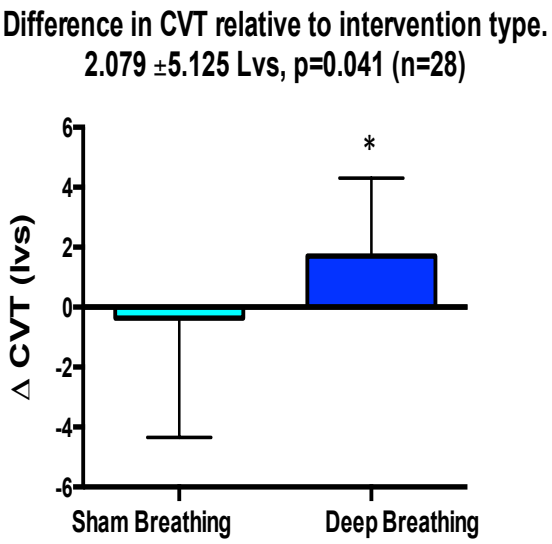


Figure 75 The comparison of the difference in ANS change between Sham breathing visit and Deep breathing, with (A) showing the change in SCR (SNS) and (B) the change in CVT (PNS).

4.3.6 Psychological Profiling Data for study 2

4.3.6.1 BFI questionnaire

The BFI questionnaire, using cumulative percentages, was used to analyse and interpret the cohort's personality domain data. The personality domains of this cohort of healthy volunteers indicated that they were evenly grouped between introversion and extroversion. (Figure 76(B)) Their agreeableness was just below the 50th percentile, which is higher than observed during study 1. (Figure 76(A)) Their conscientiousness, neuroticism and openness were above the 50th, but below the 80th percentiles and similar to that of study 1.

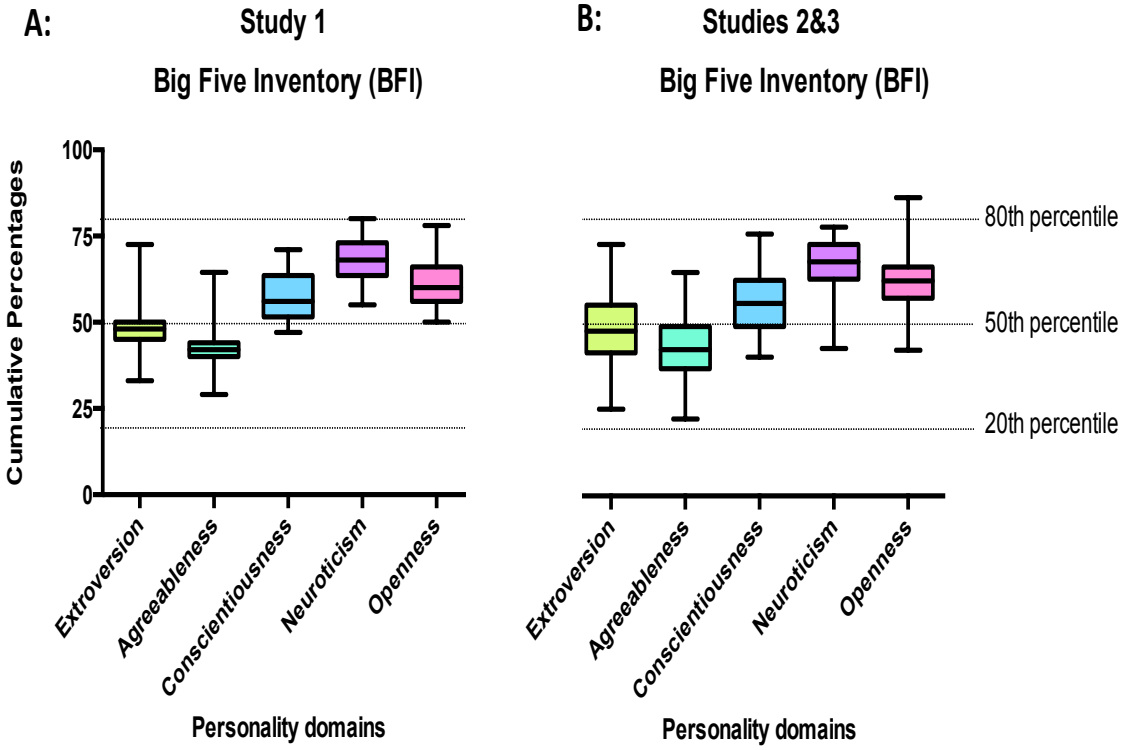


Figure 76 *Big Five inventory (BFI) as percentage of maximum possible (Cumulative Percentages) scores in (A) study 1(n=1) and (B) studies 2&3 (n=49), across the five different personality domains.*

Their Openness was slightly higher than observed in the pilot study, reflecting the need for a greater sense of 'adventure/risk-taking' by the

general public (40%) in participating with an invasive medical study compared to study 1 (15%). There were no personality extremes detected (above the 80th, or below the 20th percentile – indicative of personality disorders). As in study 1, the mean Neuroticism score, a vulnerability factor, was the highest, with openness, a protective personality factor, the second highest. Based on the BFI average response the cohort as a whole could be described as a "semi-social, slightly reserved, organized, emotionally sensitive but curious and adventurous" group. This personality description is similar to that of the pilot study, as the participants were of a similar background, education and socio-economical status. It is consistent with the self-selection that occurs with advert recruitment in the same geographical site, as only the individuals with a high degree of openness (curiosity and adventurousness) and extroversion will volunteer for this type of study.

4.3.6.2 Hospital Anxiety and Depression questionnaire

On the Hospital Anxiety and Depression Score (HADS) the mean values for anxiety, 8.97 ±2.52(SD) and depression, 8.55 ±1.52(SD) were within the borderline range (HADS score of 8-10/21), but below the clinical 'caseness' cut-off (HADS score of ≥11/21). (266) Only 16.1% of subjects met the criteria for moderate anxiety and 9.7% for moderate depression, which is below average expectation. This is reflective of the efficacy of exclusion criteria used during recruitment, as none of the subjects attracted a formal psychiatric diagnosis and as such were not on any psychotropic medication. These individuals are examples of the upper end of the normal range of a healthy cohort, with a high percentage being university students. Hence the sensitiser cohort was slightly anxious, but representative and consistent with expected means for age and gender of the general population.

4.3.6.3 State and Trait Anxiety Inventory questionnaire

Analysis of the State and Trait Anxiety Inventory (STAI) indicated firstly that the cohort's trait anxiety, 38.45 ±9.56(SD) is consistent with the expectations from a general population (38.69 ±10.34(SD)). (267) A second finding that was consistent with observations in study 1, was that the subjects' state anxiety reduced on the subsequent visit (mean ΔSTAI-S = -3.81 ±9.43(SD) per visit), and is an example of exposure habituation. There was no relationship between STAI-trait and T0 thresholds, nor with degree of acid sensitisation at subsequent time points (p= 0.84 – 0.89, linear regression). A significant negative correlation was found between STAI-trait and change in CVT during deep breathing, coeff. -1.05 (CI -0.54 to +1.44), p= 0.004, but not sham breathing, coeff. 0.45 (CI -1.73 to -0.36), p= 0.36.

4.3.6.4 Vulnerable Attachment Style Questionnaire

With the Vulnerable Attachment Style Questionnaire (VASQ), 40% of subjects had significant attachment vulnerability, which is similar to the proportion among other non-patient samples (293, 294). Of these subjects with attachment vulnerability 67% of anxious-preoccupied, and 33% of dismissive-avoidant types. The combined attachment vulnerability for the whole cohort (study 2&3) was 37%. (Figure 77)

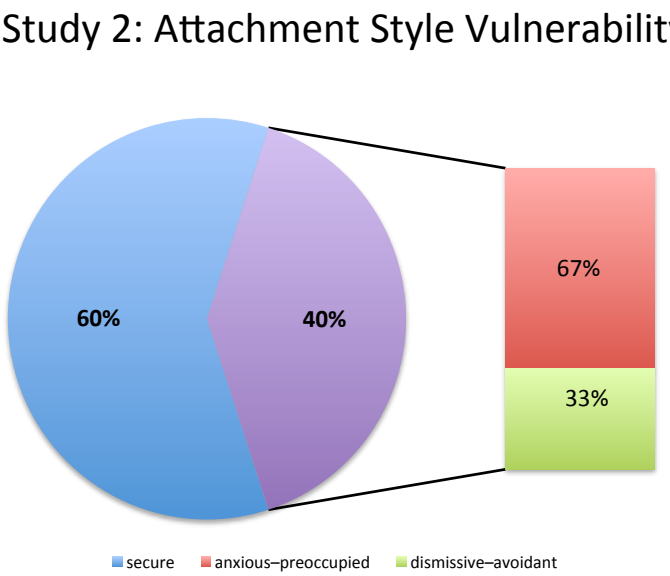


Figure 77 The Vulnerable Attachment Style Questionnaire (VASQ) findings for study 2, showing the secure/insecure percentages with the pie graph, and a brake-down of the types of insecure attachment style on the adjacent bar chart.

4.3.7 Correlation Data for study 2

During sham breathing a negative correlation between the differences in PT and SCR (yellow graph, figure 78) was detected and a positive correlation with the difference in MBP (red graph, figure 78). There was no correlation with CVT (blue graph, figure 78).

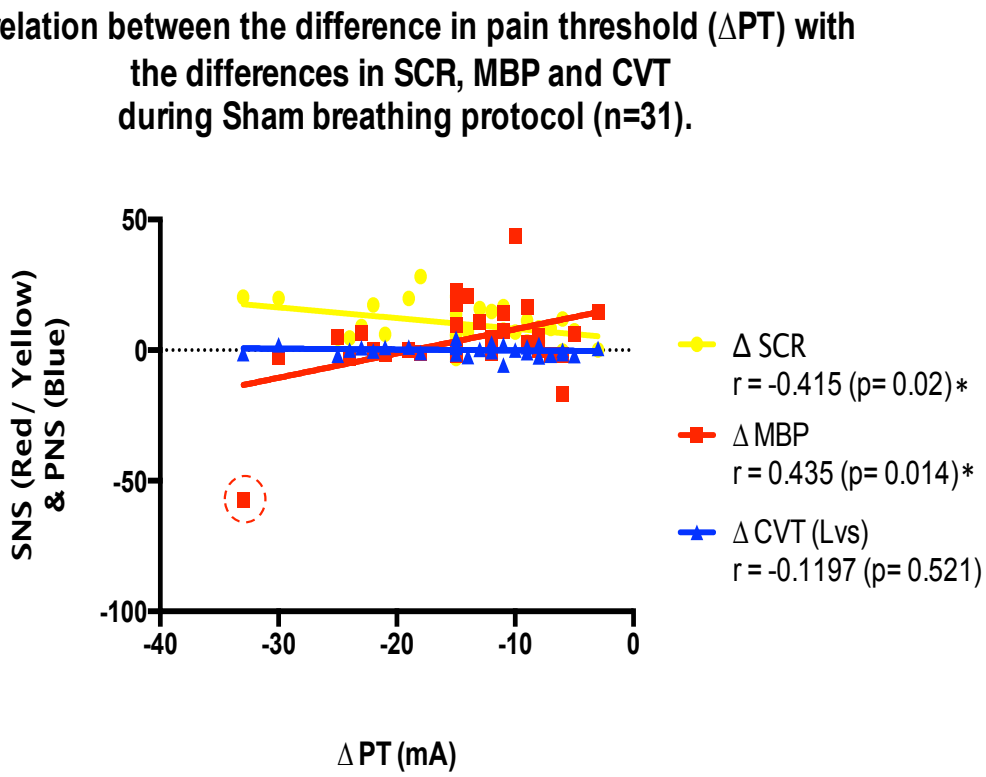


***Figure 78** The correlation between the difference in pain threshold (ΔPT) and skin conductance response (ΔSCR), blood pressure (ΔMBP) and efferent PNS (ΔCVT) during sham breathing. [Encircled: outlying point]*

This is potentially contradictory, as it implies that visceral sensitivity (ΔPT) increases with the increase in sympathetic outflow, except for the positive correlation with MBP. The ΔPT/ΔMBP correlation was sensitive to the inclusion/exclusion of an outlying point. When the analysis was repeated after the exclusion of the outlier the strength of the correlation and the statistical significance disappeared, r=0.158 (p=0.402), and suggests no contradiction, but rather a probable chance finding.

During deep breathing contrary to the above 'sham breathing-observation' (yellow graph, figure 78), the SNS now had a weak non-significant negative correlation with the mean pain threshold (SCR, yellow graph, figure 79), while the PNS had a significant positive correlation. (CVT, blue graph, figure 79)

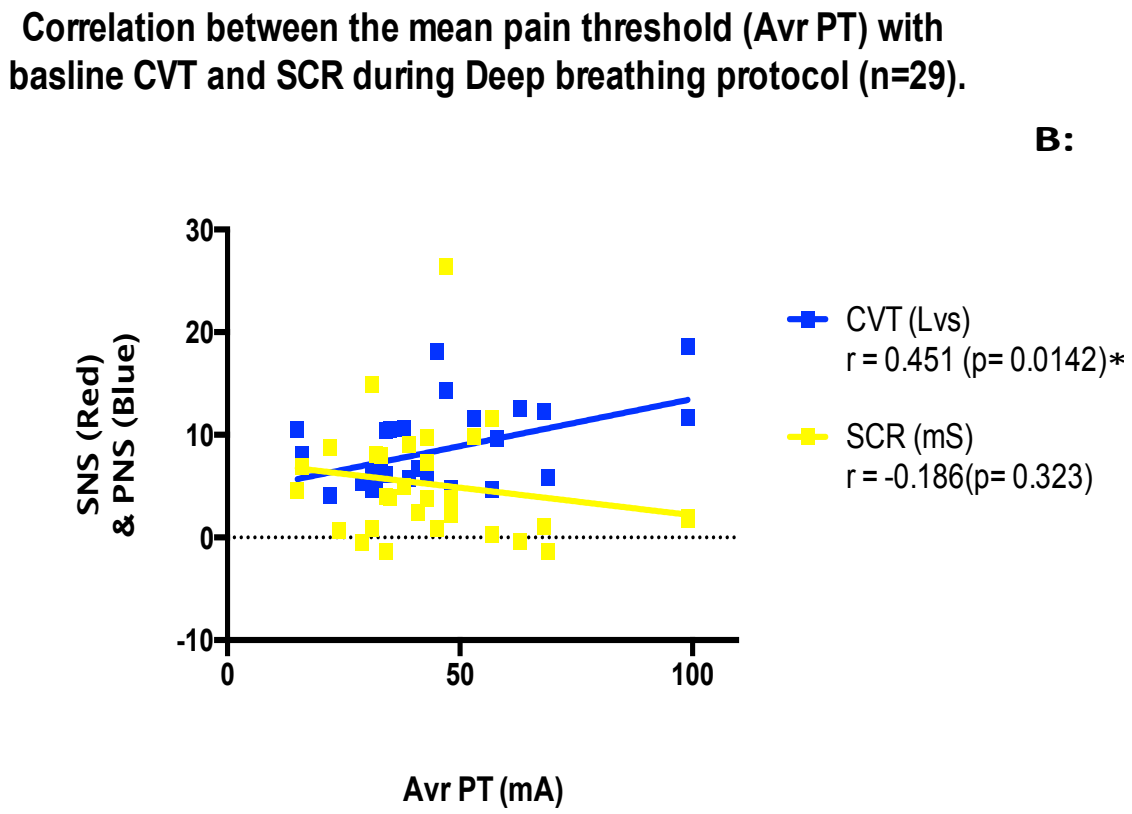


Figure 79 The correlation between the average pain threshold (Avr PT) and cardiac vagal tone (ΔCVT) during deep breathing visit.

There was a moderate positive correlation between the degree of PNS activation (ΔCVT) and BFI-conscientiousness score (red graph, figure 80).[14] Also observed during sham breathing, was a negative correlation with WAI-defensiveness (blue graph, figure 80).[14] This is of interest, as it infers that the "more" conscientious and the "less" defensive the subject is, the higher the observed CVT activation; suggestive of a 'generally more relaxed, prepared, non-threatened' attitude being associated with a higher protective vagal tone.

[14] Bonferroni correction was not used for these observations.

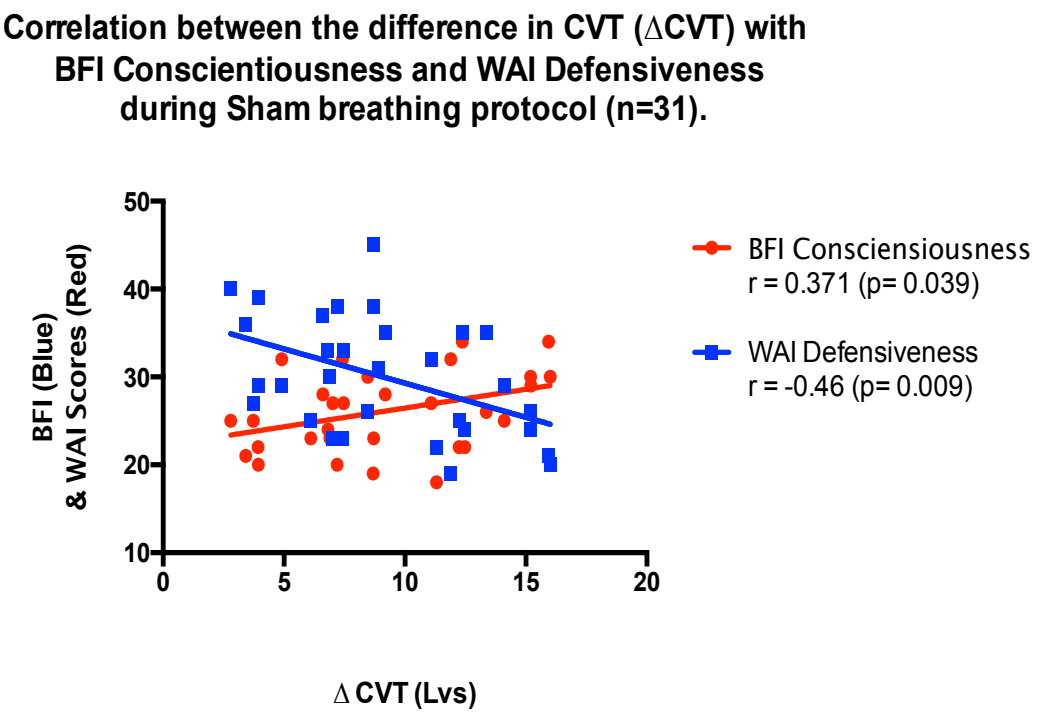


Figure 80 *The correlations between the difference in cardiac vagal tone (ΔCVT), the personality trait Conscientiousness (BFI) and WAI-defensiveness score (WAI) during sham breathing protocol.*

4.3.8 Demographic Data for study 3 (Stress induction in Non-sensitisers)

From the full cohort of 55 healthy volunteers, 18 subjects (13, male) did not sensitise to acid infusion during the Sham breathing protocol visit, and were recruited to study 3. (Figure 81) The age range was from 19-45 years with a mean age of 27 ±7.33 years. There were no obese or underweight subjects and the average body mass index (BMI) was 22.5 ±1.98kg/m^2. The subjects were recruited from different ethnic backgrounds reflective of local ethnic diversity. The majority of subjects were Caucasians (78%) followed by Asians (17%) and Africans (5%). All were acid infusion naïve.

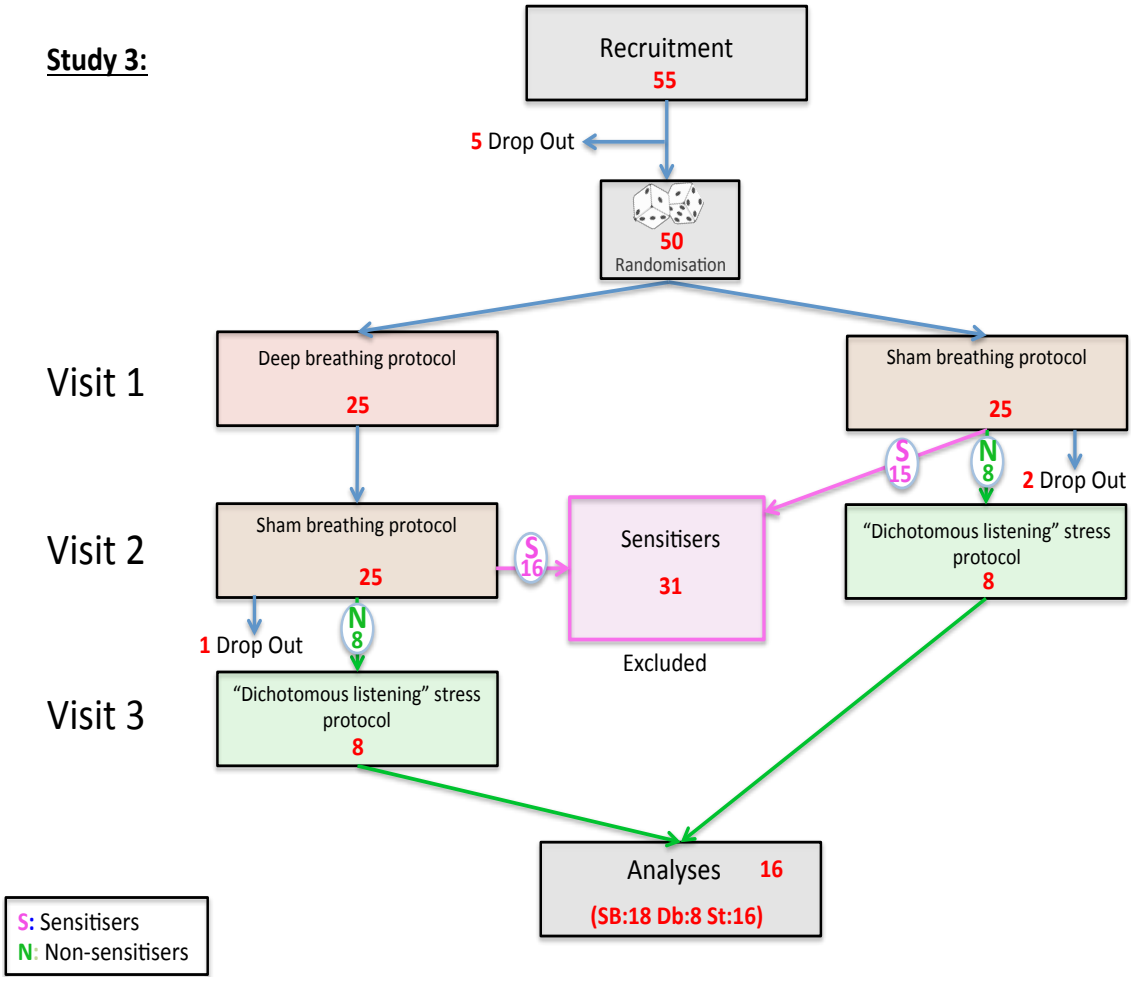


Figure 81 ***Flow diagram illustrating the final numeric outcome of participants in study 3. The experimental study design was that of a prospective randomised placebo controlled two-tiered crossover double-blinded longitudinal cohort study. [SB: Sham Breathing, St: Stress Test, Db: Deep Breathing.]***

During their first randomisation visit, five subjects could not tolerate prolonged nasal intubation even though intubations were successful and were excluded. For the final analysis, 18 subjects (13 male) completed the sham breathing protocol and only 16 the psychological stress induction protocol, as a further three subjects were unable to complete the study (due to study unrelated reasons, one had a family emergency and the other two, sudden unscheduled commitments). (Figure 81) Due to the sensitisation state 'blinded' randomisation, 8 non-sensitising

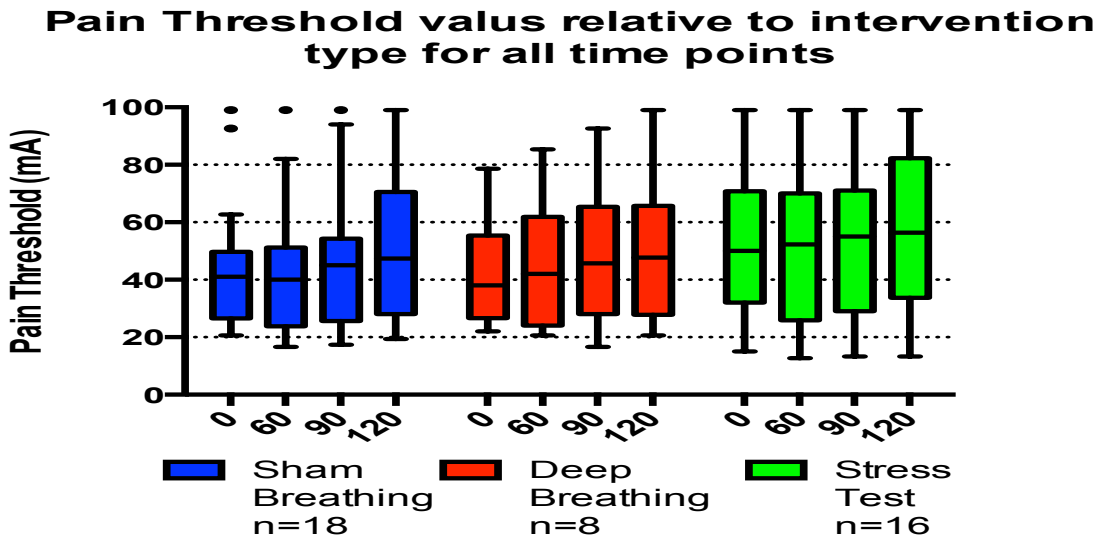
subjects completed the deep breathing protocol, before they could be identified and recruited to study 3.

4.3.9 Pain Tolerance Threshold Data of Proximal Oesophagus for study 3

4.3.9.1 Main modulation group

Absolute threshold data for the proximal oesophagus at (T0) and post-acid infusions (T60, T90, T120) for all modulation types are shown in Figure 82(A&B).

A:



B:

	T0	T60	T90	T120	T0	T60	T90	T120	T0	T60	T90	T120
Mean (mA)	44.39	42.72	45.83	50.94	42.88	45.13	48.38	50.75	54.5	51.19	54.19	56.63
Std. Deviation (SD)	22.42	22.11	23.32	26.14	18.98	21.81	24.17	25.15	25.56	26.56	26.9	27.44

Table 6

Figure 82 *(A) Graph and (B) table, illustrating and listing absolute values for proximal oesophageal pain thresholds before (T0) and after (T60 T90 and T120) acid infusion with sham, deep breathing and stress induction protocols.*

The mean individual 'pre/post-acid infusion' changes in pain thresholds (ΔPT) for all subjects in the proximal oesophageal, during sham-, deep

breathing and stress test, with the mean group value (SD) for each time point, are shown in figure 83 (A, B & C). During the stress test visit however 25% (n=4) of subjects sensitised (i.e. mean ΔPT ≤-6mA) to acid infusion, and are indicated by means of the pink rectangles, in figure 83(C).

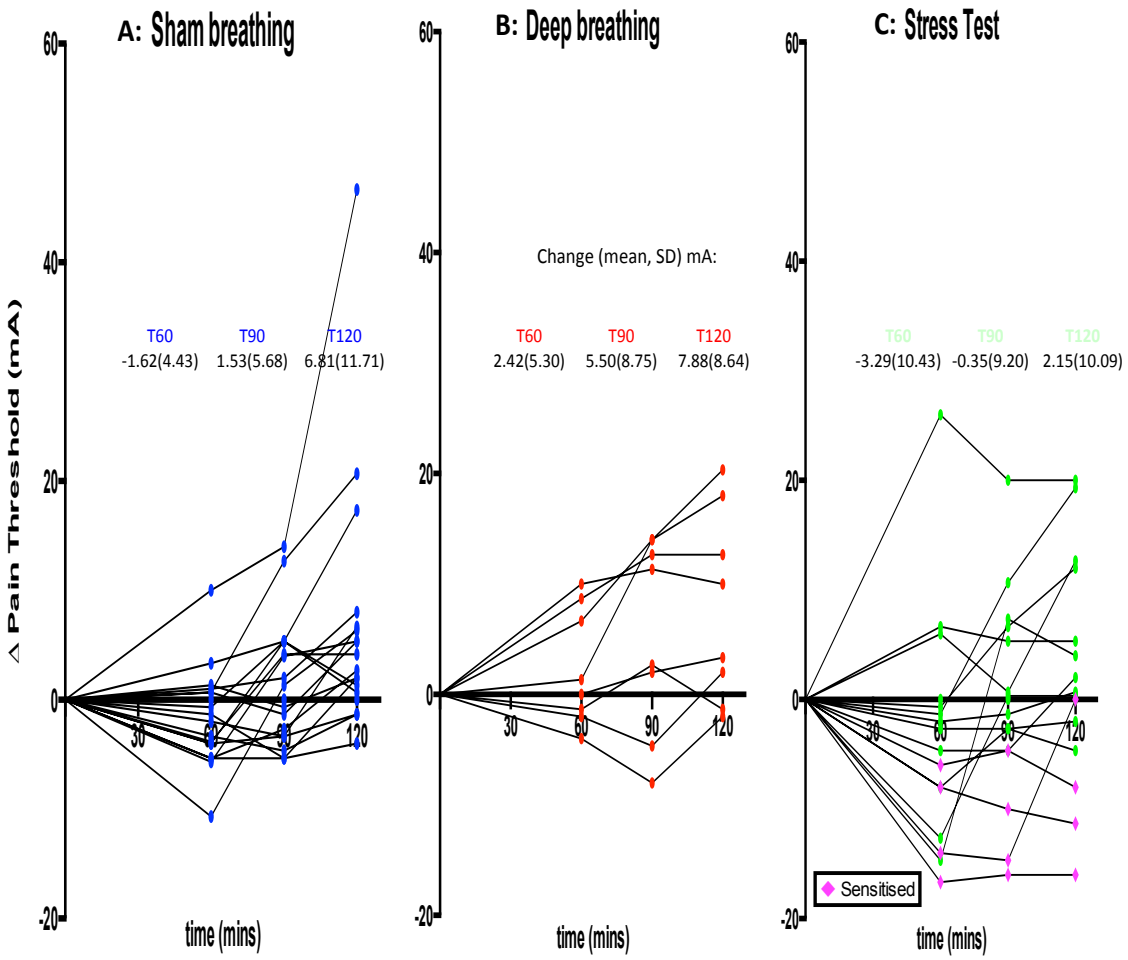


Figure 83 ***Individual values of change for proximal oesophageal pain thresholds (ΔPT) for time points T60, T90 and T120, following acid infusion with (A) sham breathing, n = 18 (B) deep breathing, n = 8 and (C) Stress Test, n = 16.***

Two-way MANOVA analyses, comparing the influence of modulation type, across all time points, showed no statistical significance between modulations for the study group as a whole. (Figure 84(A))

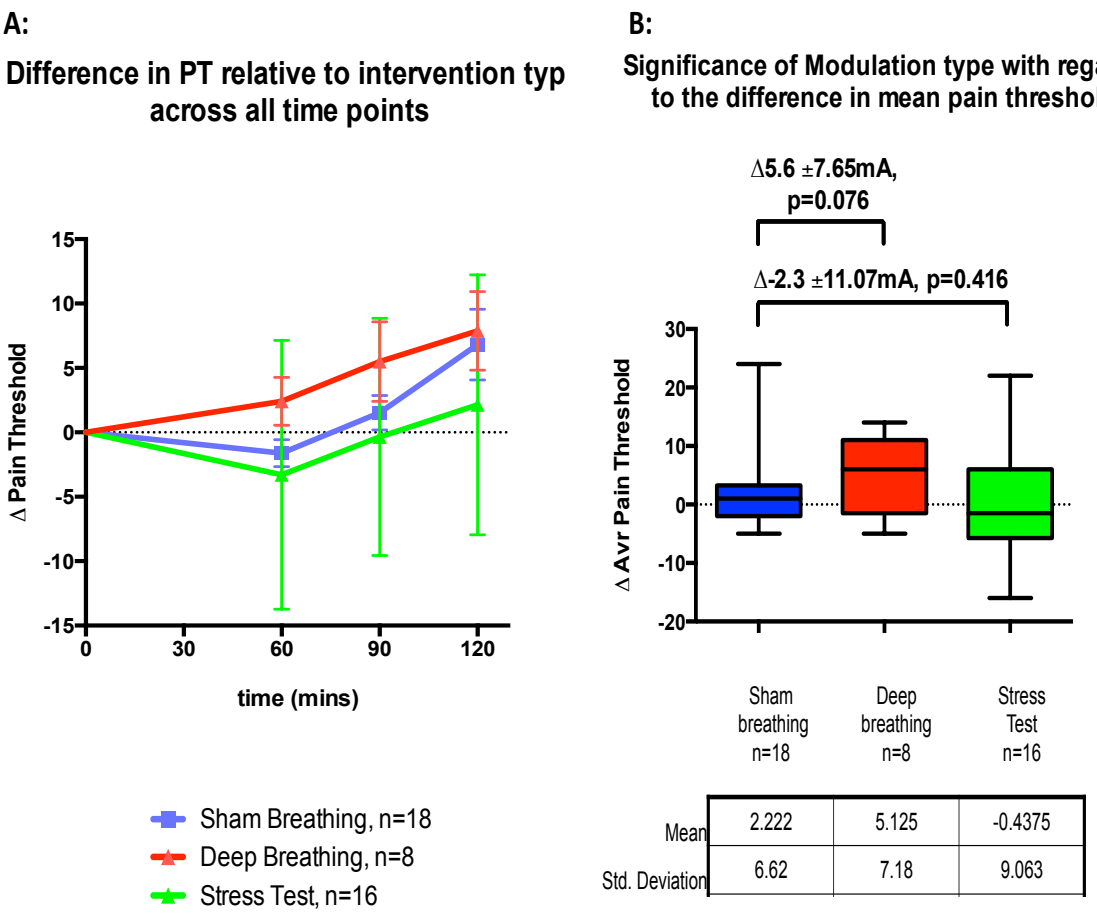


Figure 84 A: shows the difference in mean pain threshold (ΔPT) in mA, for the proximal oesophagus between baseline and the three-time points (minutes) after acid infusion, for the different modulation types. B: Showing the difference in average means of pain threshold (Δ Avr PT) in mA, for the proximal oesophagus between pre & post acid infusion, for the different modulation types.

4.3.9.2 Sensitised stress-test modulation group

Concerning the subjects that sensitised to acid infusion during the psychological stress induction visit, two-way MANOVA analyses indicated significance with regard to degree of sensitisation, contributing 25% of change over all time points (Figure 86(A)) and means comparison of the difference in pain threshold (ΔPT) post-acid, also achieved significance. (Figure 86(B))

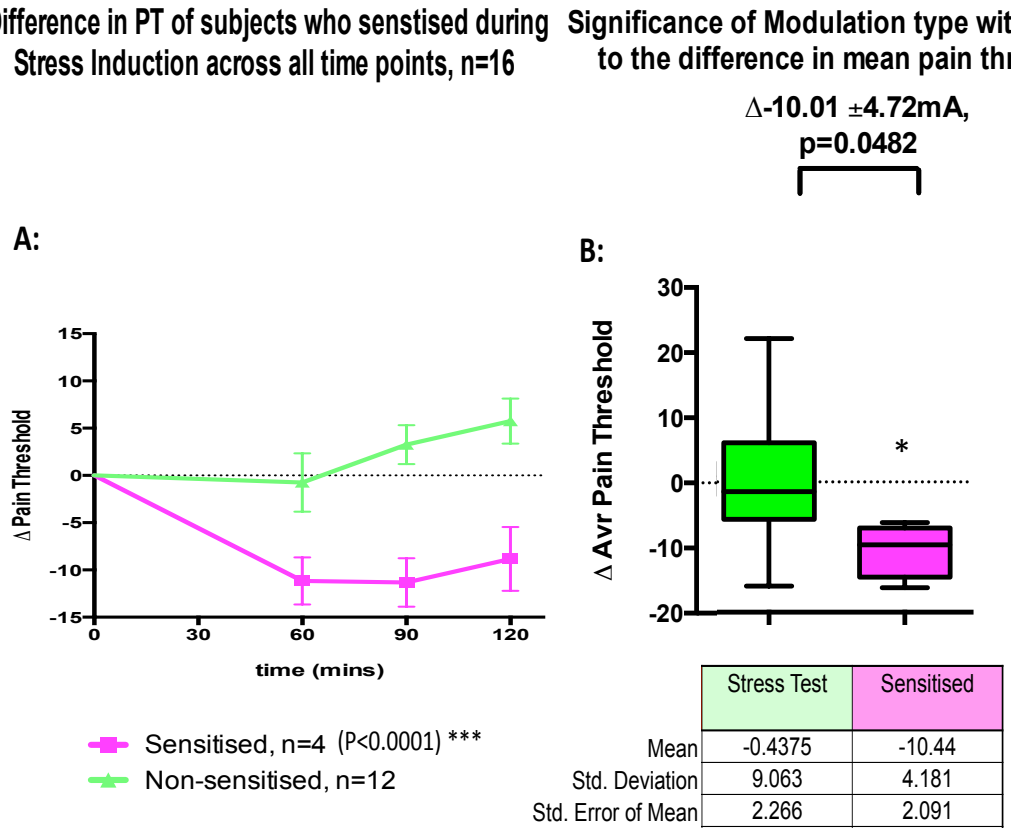


Figure 85 A: shows the difference in mean pain threshold (ΔPT) in mA, for the proximal oesophagus between sensitisation status and the three-time points (minutes) after acid infusion, for the stress induction modulation. B: Showing the difference in average means of pain threshold (Δ Avr PT) in mA, for the proximal oesophagus between pre & post acid infusion, for the different modulation types.

4.3.10 Pain Tolerance Threshold Data of Foot for study 3

The foot pain threshold data showed that both deep breathing and stress induction modulations caused no significant change with regard to sham breathing. There was thus no indication of any degree of sensitisation with regard to the somatic control (foot) demonstrated in this instance for both MANOVA analyses (Figure 73), and on average PT means comparison of pre/post-acid differences (Δ Avr PT, not illustrated). For all MANOVA analysis data stets were of similar in variance and found to be statistically matching.

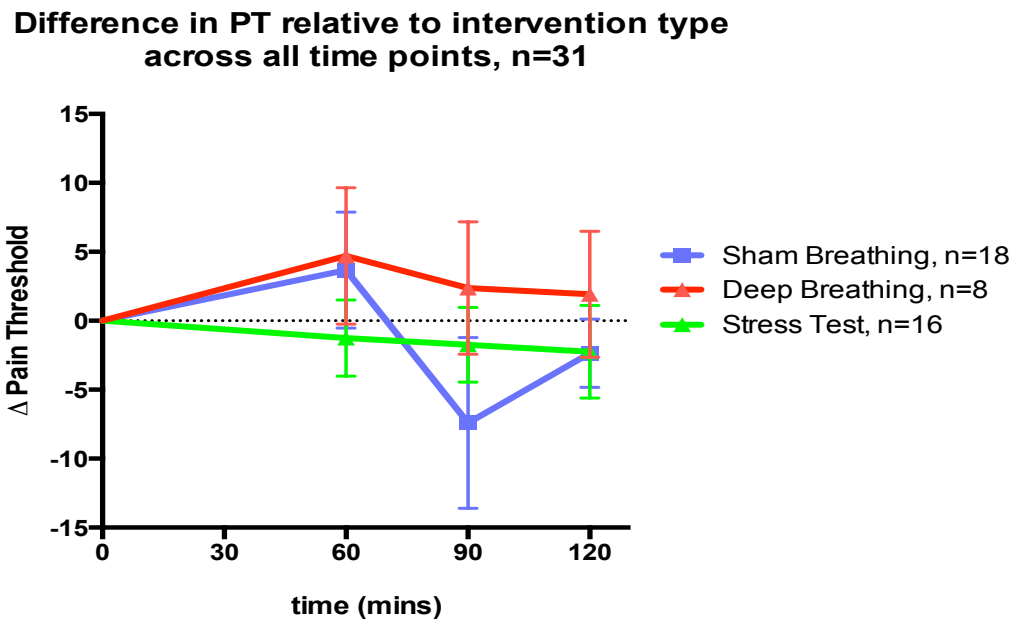


Figure 86 Shows the difference in mean pain threshold (ΔPT) in mA, for the foot between baseline and the three-time points (minutes) after acid infusion, for the different modulation types. B: shows the two-way MANOVA analysis tables across all time points for each individual modulation compared to sham breathing visit.

3.4.11 Autonomic Data for study 3

4.4.11.1 Sham breathing

The 'pre/during-acid' infusion changes in ANS for the sham-breathing visit, served as 'baseline' to which deep breathing and stress induction's modulated ANS-changes were compared; and are illustrated below in figure 87(A) & table 7(B). The changes observed during the sham breathing protocol demonstrated a post-acid increase in SNS activation, but in this group, as opposed to the sensitisers' response in study 2 (Figure 74(A&B)), there is still a degree of coinciding increased PNS activation observed. The SNS is hence still homeostatically 'apposed' to a degree, which is a novel finding not previously observed. (Shaded graph below, figure 87(A) & table 7(B).

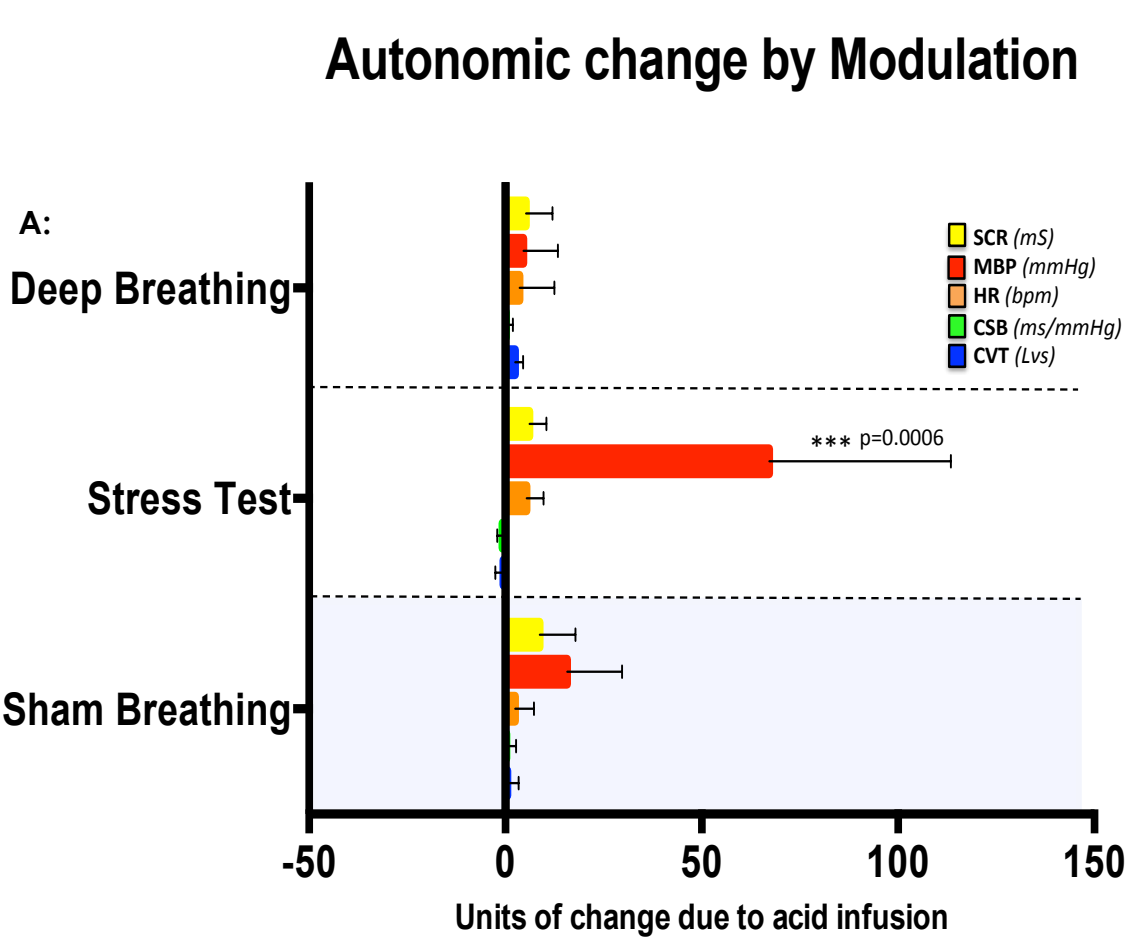


Table 7

B:

Modulation Protocol	ANS Measure	A Avr	SD	Difference between means	P value
Deep Breathing	SCR (mS)	5.28	6.74	1.07 ± 7.77	0.708
	MBP (mmHg)	4.57	8.83	-10.2 ± 12.36	0.099
	HR (bpm)	3.58	8.86	2.63 ± 10.15	0.553
	CSB (ms/mmHg)	0.2	1.75	0.117 ± 3.56	0.939
	CVT (Lvs)	2.5	2.01	2.67 ± 2.70	0.0602
Psychological Stress	SCR (mS)	6.17	4.31	-2.56± 11.64	0.3774
	MBP (mmHg)	67.22	46.22	52.01 ± 48.34	0.0006
	HR (bpm)	5.41	4.31	3.13 ± 7.08	0.097
	CSB (ms/mmHg)	-0.76	1.3	-0.94 ± 3.13	0.246
	CVT (Lvs)	-0.5	2.03	-1.13 ± 3.78	0.253
Sham Breathing	SCR (mS)	8.73	9.09		
	MBP (mmHg)	15.71	14.02		
	HR (bpm)	2.47	4.78		
	CSB (ms/mmHg)	0.29	2.45		
	CVT (Lvs)	0.53	2.83		

Figure 87 The comparison between the different 'pre/post-acid infusion' ANS changes between sham breathing (shaded), deep breathing and stress test modulation-types, are graphically illustrated in (A); with table (B) below showing the mean values of change & standard deviations for each specific protocol, and for deep breathing's comparison & p-value significance with regards to sham breathing. [Abbreviations are as follows; SCR: skin conductance response, MBP: mean blood pressure, HR: heart rate, CSB: cardiac sensitivity to baroreflex, & CVT: cardio vagal tone.

4.4.11.2 Deep breathing

The changes observed during the deep breathing protocol (figure 87(A) and table 7(B)), demonstrated no change post-acid/modulation in sympathetic outflow, but showing a further increase in the para-sympathetic activation. Of note is that although the CVT showed a strong trend, there was no statistical difference to that observed during sham breathing. Interestingly the non-sensitisers' PNS co-activation is consistent for both deep-and-sham breathing visits.

4.4.11.3 Stress test

The changes observed during the psychological stress test induction (figure 87(A) and table 7(B)), demonstrated a strong statistical significant post-acid/modulation increase in the MBP part of the sympathetic outflow, with a small withdrawal of para-sympathetic activation (a novel finding in non-sensitisers). Looking at the ANS response in the non-sensitiser study group as a whole, in spite of the SNS increase and the slight PNS withdrawal (their only example of ANS non-co-activation), the modulation was not sufficient in producing an associated increase in post-acid sensitisation in the group, except in the 25% of individual subjects who sensitised (Figure 83), that we will turn to next.

4.4.11.4 Sensitised vs. non-sensitised

Comparing the ANS responses of subjects who sensitise vs. those that did not, as illustrated in figure 88(A&B) below. The changes observed during the psychological stress test induction demonstrated a significantly larger activation of the SNS (SCR - figure 89(A)) by the non-sensitisers, while the PNS (CVT - figure 89(B)) was not statistically significant.

A:

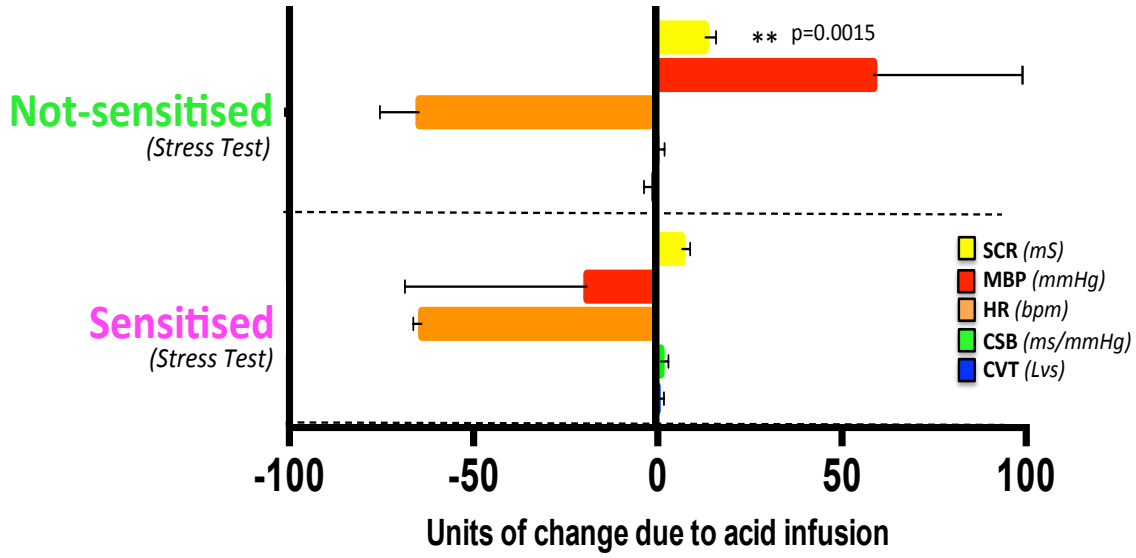


Table 8

B:

Modulation Protocol	ANS Measure	A Avr	SD	Difference between means	P value
Not-sensitised (Stress Test)	SCR (mS)	13.61	2.96	6.372 ± 1.626	0.0015
	MBP (mmHg)	59.65	302.50	78.35 ± 155.5	0.6229
	HR (bpm)	-64.58	10.63	-0.6818 ± 5.481	0.9029
	CSB (ms/mmHg)	0.16	2.33	-1.361 ± 1.321	0.3216
	CVT (Lvs)	-0.18	2.93	-0.6318 ± 1.585	0.6966
Sensitised (Stress Test)	SCR (mS)	7.24	2.23		
	MBP (mmHg)	-18.70	49.73		
	HR (bpm)	-63.90	2.24		
	CSB (ms/mmHg)	1.53	2.03		
	CVT (Lvs)	0.45	1.85		

Figure 88 The comparison between the different 'pre/post-acid infusion' ANS changes between the mean difference in ANS change between the group of subjects who sensitised and who did not, during the psychological stress protocol, are graphically illustrated in (A); with table (B) below showing the mean values of change & standard deviations for each specific protocol, and for deep breathing's comparison & p-value significance with regards to sham breathing. [Abbreviations are as follows; SCR: skin conductance response, MBP: mean blood pressure, HR: heart rate, CSB: cardiac sensitivity to baroreflex, & CVT: cardio vagal tone.

Of note are two potentially contradictory observations regarding the *sensitised* 'non-sensitiser' group's observed ANS responses:

(i) Opposite to the observations in study 2, here the *sensitised* 'non-sensitisers' demonstrate no PNS withdrawal (possibly even a trend indicative of PNS increase), yet sensitised. In study 2, CVT had a positive correlation with increased pain threshold (Avr PT), and hence a higher CVT would be associated with desensitisation. (Figure 79)

(ii) Compared with the *non-sensitised* 'non-sensitisers' who were <u>not</u> able to maintain ANS co-activation, the sensitised group had a statistically significant lower degree of SCR activation, yet sensitised. In study 2, SCR correlated positively with increased pain sensitivity (ΔPT), and hence a lower SCR would be associated with desensitisation. (Figure 78)

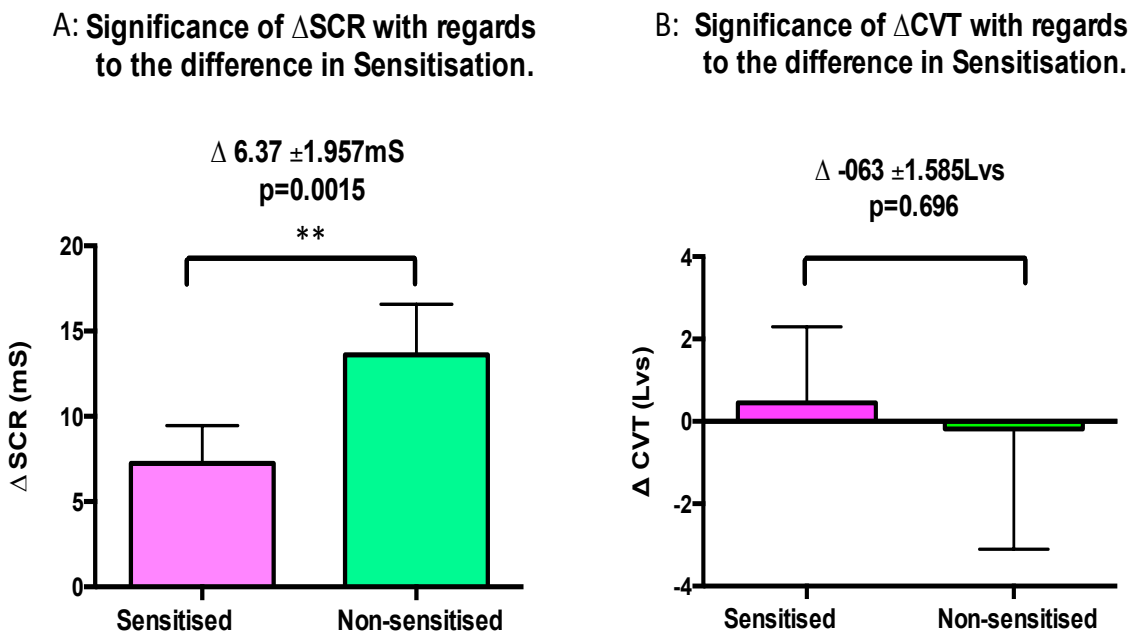


Figure 89 The comparison of the difference in ANS change between the group of subjects who sensitised (pink) and who did not (green) during the psychological stress protocol with (A) showing the change in SCR (SNS) and (B) the change in CVT (PNS).

4.4.12 Psychological Profiling Data for study 3

4.4.12.1 BFI questionnaire

The BFI questionnaire, using cumulative percentages[15], was used to analyse and interpret the cohort's personality domain data. A comparison of personality domains of the cohort of healthy volunteers between study 2 and 3, indicated that they were both evenly grouped between introversion and extroversion, with the sensitisers slightly more extrovert and higher for agreeableness and conscientiousness. (Figure 4.24(B)) The non-sensitisers scored higher for neuroticism and openness. (Figure 4.24(A)) The respective profiles suggest that the sensitisers are more 'out-going' and 'eager to please', with the non-sensitisers more 'reserved and conservative' yet 'adventurous'. Both groups were similar in general personality, reflecting the confounder of self-selection for voluntary experimentation.

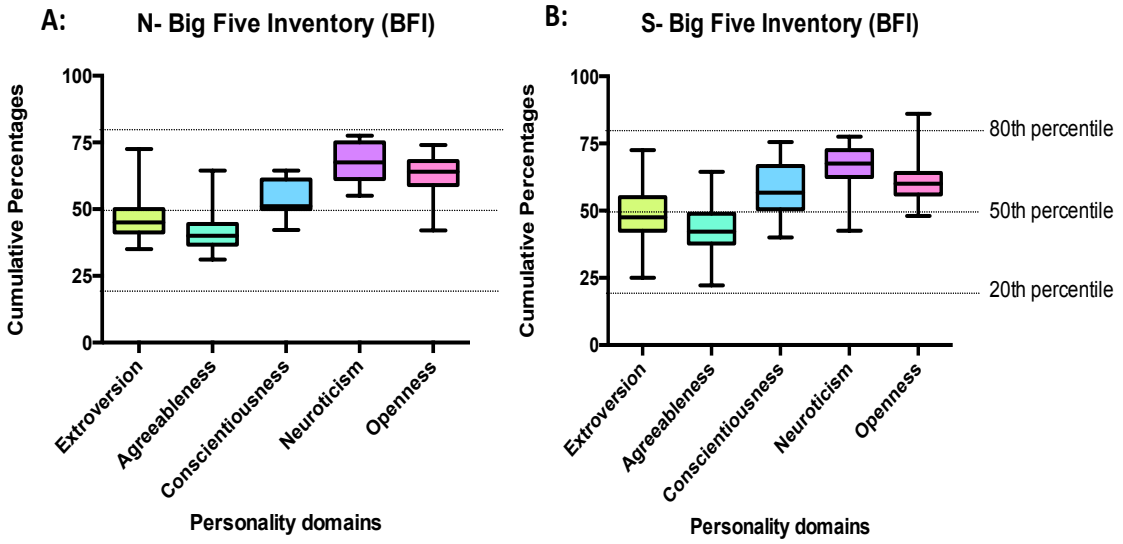


Figure 90 Big Five inventory (BFI) as percentage of maximum possible (Cumulative Percentages) scores in (A) studies 3, non-sensitisers and (B) study 2, sensitisers, across the five different personality domains.

[15] For a more detailed description see explanatory note, appendix thee, and chapter 3, section 3.4.4, page 135).

4.4.12.2 Hospital Anxiety and Depression questionnaire

On the Hospital Anxiety and Depression Score (HADS) the mean values for anxiety, 8.44 ±1.76(SD) and depression, 9.55 ±1.73(SD) were within the borderline range (HADS score of 8-10/21), but below the clinical 'caseness' cut-off (HADS score of ≥11/21). (266) Only 11.1% of subjects met the criteria for moderate anxiety and 16.7% for moderate depression, which is below average expectation. The sensitisers (study 2) scored less for depression (9.7%) and more for anxiety (16%). The opposite was observed for the non-sensitisers (study 3) (16.1% - anxiety & 11.1% - depression). This could suggest that higher anxiety states (sensitisers) coincided with a greater awareness and sensitivity to influence from internal/external environmental stimulus, while not the case for depression (non-sensitisers). (295)

4.4.12.3 State and Trait Anxiety Inventory questionnaire

Analysis of the State and Trait Anxiety Inventory (STAI) indicated firstly that the cohort's trait anxiety (STAI-T), 34.33 ±8.05(SD) is below general population expectations (38.69 ±10.34(SD)). (267) A second consistent finding was that the subjects' state anxiety reduced with each subsequent visit, and is an example of exposure habituation. Between visit 1 and 2 state anxiety dropped by Δ-4.49, and between visit 1 and 3, by a further Δ-2.44. (mean ΔSTAI-S = -3.47 ±5.19(SD) per visit)

In comparing STAI-S analysis with regard to *non-sensitised* 'non-sensitisers' (non-sensitised-ns) vs. *sensitised* 'non-sensitisers' (sensitised-ns), the sensitised-ns had a lower trait anxiety, 29.00 ±2.16(SD) compared to the non-sensitised-ns: 35.17 ±8.20(SD), p=0.504; replicating this previously observed contradictory finding. (296) When the sensitised-ns & non-sensitised-ns's 'anxiety habituation' were compared, the sensitise-ns

habituated more, mean ΔSTAI-S(S) = -2.25 per visit, than the non-sensitisers-ns, mean ΔSTAI-S(N) = -0.875 per visit. At the time of the third (stress induction) visit, the sensitised-ns' STAI-S was 21.00 <0.00(SD), compared to the non-sensitisers-ns' 30.33 ±4.72(SD), p=0.038. This finding could suggest "non-adaptive calm", or coinciding conflicting emotional/arousal states, sighted by Gray and McNaughton. (66)

4.4.12.4 Toronto Alexithymia Scale questionnaire

In comparing the Toronto Alexithymia Scale (TAS-20) analysis with regard to the sensitised/non-sensitised-ns, the sensitised-ns had a significant higher TAS-20 score, 87.5 ±5.45(SD) compared to the non-sensitised-ns' 76.00 ±6.99(SD), p=0.010, replicating similar previous findings. (297-299) The "Difficulty describing feelings" TAS-20 sub-scale was specifically predictive as the non-sensitised-ns had a mean of 21.5 ±2.08(SD) compared to the non-sensitised-ns' 17.00 ±2.52(SD), p=0.006.

4.4.12.5 Vulnerable Attachment Style Questionnaire

With the Vulnerable Attachment Style Questionnaire (VASQ) 33% of subjects had significant attachment vulnerability, which is lower than expected for a non-patient population (293), of which 67% was of anxious-preoccupied, and 33% of dismissive-avoidant types. The percentage of significant attachment vulnerability for the non-sensitisers (study 3 - 33%) was less than for the sensitisers (study 2 - 40%), as well as less for the cohort as a whole (studies 2&3 - 37%). (Figure 91)

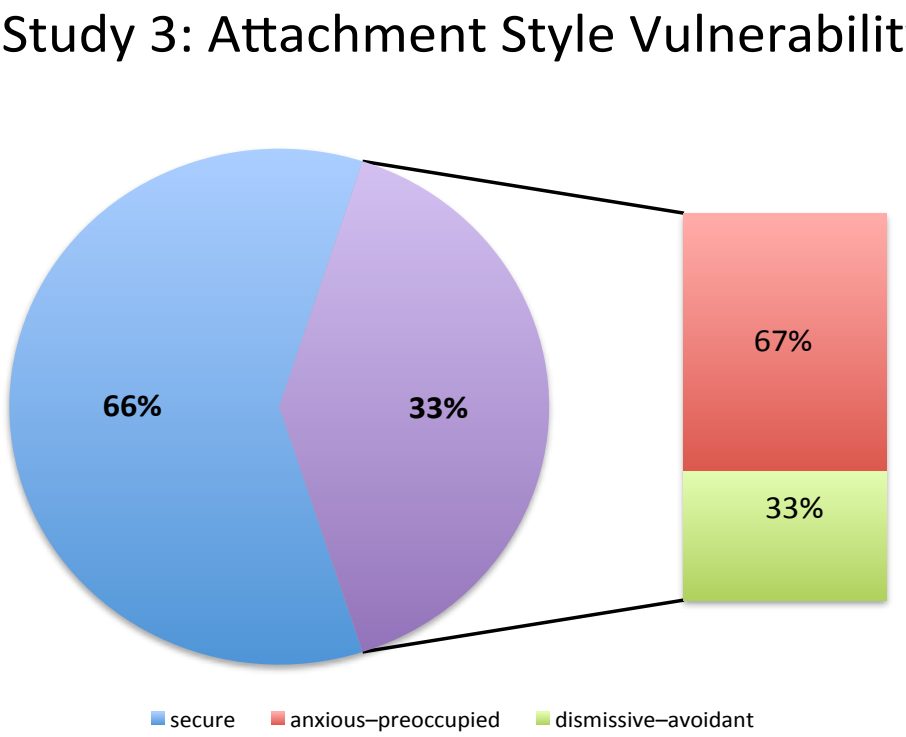


Figure 91 ***The Vulnerable Attachment Style Questionnaire (VASQ) findings for study 3, showing the secure/insecure percentages with the pie graph, and a brake-down of the types of insecure attachment style on the adjacent bar chart.***

In comparing the VASQ analysis with regard to the sensitised/non-sensitised-ns sub-group, the sensitised-ns had significant higher attachment vulnerability VASQ percentage of 50%, which is also higher to the proportion when compared to other non-patient samples. The non-sensitised-ns' 33%, on the contrary was lower than expected for a non-patient population. (293, 294) (Figure 92) The non-sensitised-ns' vulnerability was consistent with that of the cohort (33%), but the sensitised-ns were 17% higher (50%).

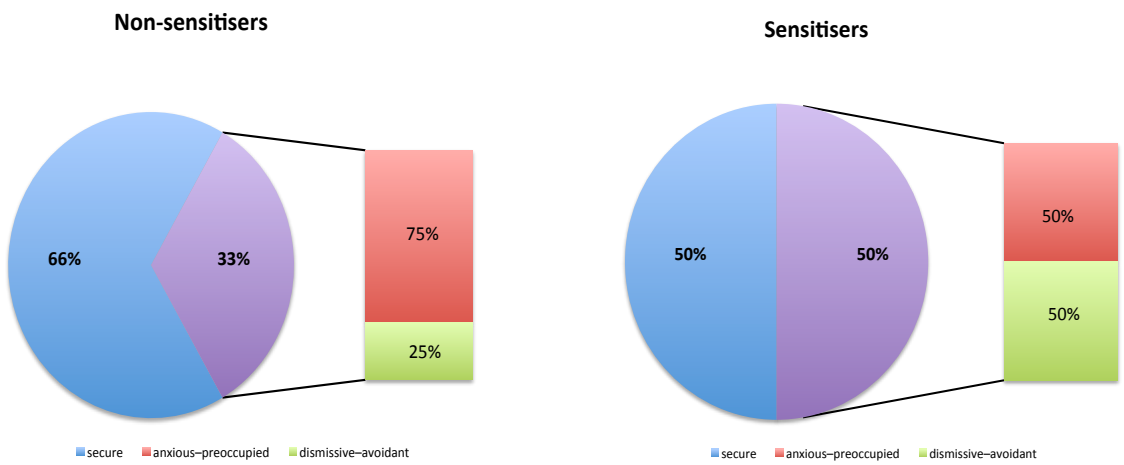


Figure 92 *The Vulnerable Attachment Style Questionnaire (VASQ) findings, showing the secure/insecure percentages with the pie graph, and a brake-down of the types of insecure attachment style on the adjacent bar chart.*

4.4.13 Correlation Data for study 3

During sham breathing visit a positive correlation between pain threshold (Avr PT) and CVT was detected, r=0.518 (p=0.032), i.e. the higher the CVT, the higher the PT. This replicates the finding that visceral pain threshold increases with the increase in para-sympathetic outflow. (132, 133) (Figure 93)

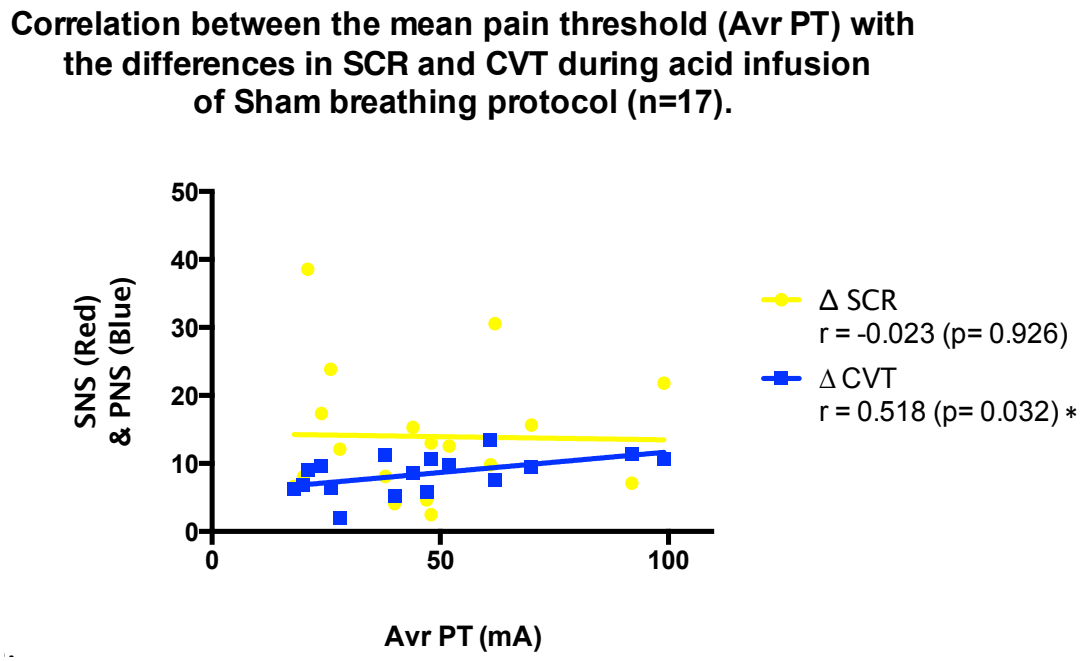


Figure 93 *The correlation between the average pain threshold (Avr PT) and cardiac vagal tone (ΔCVT) during sham breathing visit.*

During psychological stress induction visit a positive correlation between the degree of pain sensitivity (ΔPT) and SCR was detected, r=0.063 (p=0.030). This indicates that visceral pain sensitivity increases with the increase in sympathetic outflow during stress. (Figure 94)

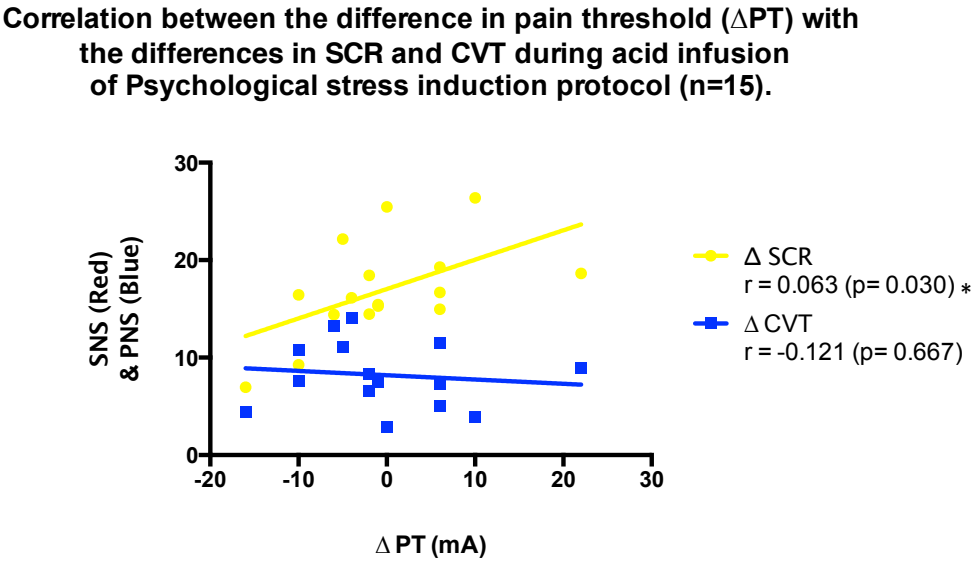


Figure 94 *The correlation between the difference in pain threshold (ΔPT) and cardiac vagal tone (ΔCVT) during deep breathing visit.*

4.5 Discussion

The results of study 2 provide evidence that visceral pain hypersensitivity, induced in the proximal oesophagus by acid infusion in the distal oesophagus, is prevented by deep breathing through its action on the increasing PNS tone.

Hitherto, the mechanisms by which deep breathing increases PNS tone have been incompletely understood. (300-302) It has been hypothesised that deep breathing increased PNS tone through its action on increasing afferent baroreflex signalling via the vagus nerve. (228) This enhances neuronal activity in the nucleus tractus solitarius (NTS), located in the caudal medulla, resulting in excitation of pathways leading to the nucleus ambiguous (NA), a structure within the medulla responsible for increasing efferent vagal tone, i.e. CVT. To date, to the best of my knowledge, this physiological reflex arc has not been demonstrated with a direct, rather than proxy, measure of CVT in humans. These results therefore provide evidence that deep breathing manoeuvres do cause a demonstrable objective rise in CVT and thus PNS tone, which could represent a physiological mechanism of action of many complementary therapies in which deep breathing is emphasised. (57, 303)

It has been in excess of 20 years since the role of vagal afferents, where the effects of vagotomy, with or without supplementary vagal nerve stimulation (VNS), in modulating pain in animal models were first reported. (304) For instance, Chen *et al.* have presented evidence to support the hypothesis that vagal afferents modulate sensorimotor responses to visceral pain emanating from the GI tract *per se*. (305) In this study, the authors' measured visceromotor responses (VMR) to graded colorectal distension (CRD) following electrical VNS, or topical application of lidocaine to the vagus nerve, following subdiaphragmatic

vagotomy in conscious rats. In both of these scenarios, a reduction of VMR to increasing CRD was noted thus indicating enhancement of PT.

Study 2 was devised to translate these findings into a human model of visceral pain in order to evaluate the importance of physiologically manipulating PNS tone in the development of central sensitisation. Central sensitisation is considered to be an important component of the endogenous pain regulatory system, whose dysfunction is considered to play a role in the maintenance of chronic visceral pain, through its functional and dynamic interaction with the ANS. (306, 307) For the first time in a model of human visceral hypersensitivity it has been demonstrated that deep breathing increases PNS tone and prevents the development of central sensitisation. However thus far, objective experimental evidence for the effectiveness of deep breathing in management of visceral pain is lacking. Nevertheless, both common personal experience and a number of studies have postulated its efficacy in ameliorating both acute and chronic somatic pain. In the context of chronic somatic pain syndromes, deep breathing has been observed to reduce pain and increase daily functioning in patients with fibromyalgia. (308) Similarly in acute somatic pain, what parent has not soothed their child, following a grazed knee for argument, with the suggestion of "*taking deep breaths,*" presumably conferring a degree of analgesia from the said injury. Somewhat more objectively, Friesner *et al*., have demonstrated breathing induced analgesia, when comparing deep breathing with natural breathing, during thoracic drain removal, although this study did not control for distraction. (309) More recently, Chalaye *et al*. resolved this confounding, evaluating basic HRV variables and thermal PT during deep breathing and distraction. (310) In healthy subjects, deep breathing increased proxy measures of vagal activity and resulted in elevated PT, and whilst distraction produced similar relative

analgesia it was not accompanied by changes in autonomic tone. In the context of this data, it would be important to study the effect of co-administered atropine and deep breathing on sensitisation. This could potentially examine and contrast the contribution and importance of the neurobiological pathways that underlie deep breathing induced PNS analgesia, with the degree of associated distraction which arguably accompanies all paced breathing techniques, and could highlight their possible difference. Distraction is a manifold phenomenon; the analgesia that was observed could plausibly be due to the observed reduction in anxiety.

Anxiety at the time of GI injury or inflammation increases the risk of developing chronic visceral hyperalgesia and symptoms. (41) Sharma *et al.* recently examined whether anxiety influences acid-induced hyperalgesia in a cohort of healthy subjects. (311) In this study the investigators demonstrated that acute anxiety induction, through autobiographical recall of adverse life events, caused an increase in sympathetic nervous system tone, with concomitant PNS withdrawal, and an increase in acid-induced oesophageal hyperalgesia. Thus anxiety may therefore facilitate the development of central sensitisation in the oesophagus presumably in combination with modulatory influences of the ANS. Study 2 evaluated trait anxiety measures and saw a small negative effect on CVT but not PT. In combination therefore, both short (state) and long (trait) term anxiety measures influence ANS tone in response to acid-infusion although the former may confer hyperalgesia possibly through the acute withdrawal of PNS tone influencing the process of central sensitisation.

Whilst this study was not designed to describe the precise neurobiological mechanisms by which increasing PNS tone imparts an

analgesic effect, data derived from VNS studies have facilitated important insights to be garnered. It maybe surmised that such mechanisms may be manifest at three levels from the periphery to central neurotransmission and supraspinal areas. Firstly, in the periphery, it has been shown that VNS may have a dual effect with seemingly inhibitory and excitatory effects below and above C3 respectively, raising the possibility that neurones arising from the propriospinal tracts of higher cervical segments may confer anti-nociception in more distal segments. (312, 313) Secondly, a plethora of neurotransmitter systems have been implicated in vagal mediated anti-nociception including serotonin, noradrenaline and opioids. (314) For example, opioid antagonism, which would be expected to cause hyperalgesia, has been shown to not influence PT following functional vagotomy thereby suggesting a role for opioidergic pathways in vagal mediated anti-nociception. (315) Vagal afferents signalling also influences the allostatic hypothalamic-pituitary-adrenal axis through indirect activation of the parabrachial nucleus through the modification of adrenocorticotrophic hormone, corticosterone and adrenaline, themselves arbitrators of inflammation and nociception. (316, 317) Finally, within the brainstem, the NTS forms part of the central autonomic network (CAN), itself encompassing a network of highly interconnected pain inhibition relays in association with the periaqueductal grey, lateral parabrachial nucleus and the ventrolateral medulla. (318) Experimental evidence, largely derived from animal studies utilising local aesthetic blockage of the constituent areas of the CAN, support the notion that these structures are the main substrates for vagal induced analgesia. (319, 320) Moreover, in humans, following short-term transcutaneous VNS, functional neuroimaging shows changes in activity in areas of the visceral pain neuromatrix, such as the limbic brain areas, including the amygdala, hippocampus and parahippocampal gyrus. (321)

There are a number of important therapeutic implications of study 2's findings. An immediately attractive suggestion is whether the direct measurement of CVT during deep breathing techniques may facilitate the objective interrogation of the success of such measures in changing PNS tone but also inducing analgesia. Furthermore, interventional manipulation of PNS tone could be used to identify patients with visceral hypersensitivity as sequelae of central sensitisation *per se*. For instance, if it were possible to show that hyperalgesia was lessened following administration of a technique that increased PNS tone, it would provide evidence for central sensitisation as the underlying cause. In contrast, failure to respond would suggest a different mechanism such as hypervigilance. Such characterisation would also allow appropriate individualisation of management to the underlying mechanism, such as cognitive behavioural treatments in those with hypervigilance and pharmacotherapy in those with central sensitisation. Equally, it is possible that episodic utilisation of deep breathing techniques during acute inflammatory episodes, such as during gastro-oesophageal reflux events, may produce both symptomatic relief and prevention of central sensitisation, hyperalgesia and chronicity of symptoms that are seen in EO. Finally with regard to study 2, transcutaneous electrical VNS stimulation has been shown to increase somatic PT and reduce pain ratings, in the absence of any demonstrable cardiovascular side effects, and therefore this novel non-invasive technology may offer a treatment option in the prevention of central sensitisation and with it, chronic pain, in patients with FGID. (322)

With the consideration of study 3's results evidence is provided that explains and clarifies the hitherto poorly understood multifactorial ANS regulatory mechanisms of visceral pain hypersensitivity in non-sensitising subjects. This group has never been studied using this model before, and

it is with the inclusion of their ANS response to acid that allows us for the first time to observe and compare the ANS response of healthy subjects across the complete spectrum of visceral hypersensitivity reactions as induced by this model.

In study 2 it has been demonstrated how increase in PNS activation (and SNS withdrawal) is associated with reversal of hypersensitisation, but it is only when compared with the responses of the non-sensitisers that a deeper understanding can be reached. The most striking observation is the contradictory finding that in non-sensitisers under stressful conditions an increase in PNS (CVT) is associated with sensitisation and activation of SNS (SCR) is now associated with non-sensitisation. This is an example of what has now come to be known as the "Vagal Paradox". (8) It is only with the incorporation of 'polyvagal' and 'attachment' theory that a more full and coherent discussion of the observed results can be given as hypothesis.

The Polyvagal theory is proposed by Porges *et al.* (8, 215, 292) and has gained great acceptance as it is increasingly supported by laboratory findings. From a psychological perspective, it provides an understanding of visceral self-regulation and sensory modulation. A phylogenetic approach is proposed to explain the vagal paradox in terms of the medullary source nuclei of the dorsal motor nucleus (DMNX) and nucleus ambiguous (NA). The term polyvagal is used as it distinguishes between the two main branches of the vagus nerve:

1: The primitive unmyelinated "Vegetative Vagus"; which originates in the DMNX, and is associated with passive reflexive regulation of visceral functions, and mediates the most primitive 'freeze' stress response, which is part of the reptilian response system.

2: The new-myelinated "Mammalian Vagus"; which originates in the medullary source of the NA. The ventral vagal complex (including NA) is related to processes associated with attention, motion, emotion and communication, and mediates the most recent evolutionary 'communication system' stress response that regulates heart (RSA) and bronchi to promote calm and self-soothing physiological states.

Physiological states support different classes of behaviour. Vagal withdrawal, for instance, would support mobilisation behaviours of 'fight-n-flight'. Vagal activation would (via NA) support spontaneous 'social engagement' behaviours by means of structural links between brainstem nuclei and the striated muscles of the face and the smooth muscles of the viscera. (99) This is known as neuroception, and is the mechanism with which defence strategies are triggered. Neuroception, as a process, determines whether specific features in the environment elicit specific physiological states that would support either 'fight-flight' or 'social engagement' behaviours. It involves areas of the temporal cortex that decode biological movement and detect the intentionality of social interactions and would distinguish them between situations that are 'safe' or 'threatening'. (118) Porges proposes that the evolution of the ANS provides an organising principle to interpret the adaptive significance of affective processes. It thus links the evolution and structure of the ANS to affective experience, emotional expression, facial gestures, vocal communication and contingent social behaviour-and-interaction. Hence a plausible explanation of socio-emotional, communication dysfunctions and visceral disregulation and sensitisation is potentially provided.

Three phylogenetic stages of vertebrate ANS development are proposed (Table 9). Each stage is associated with a distinct ANS

subsystem that is retained and expressed by mammals.[16] These ANS subsystems are phylogenetically ordered and behaviourally linked to (III) social communication (NA), (II) mobilisation (Spinal cord) and (I) immobilisation (DMNX). The three subsystems can be conceptualised as dynamic, providing adaptive responses to progressively safe, dangerous, or life threatening events and contexts.

	ANS Component	Behavioral Function	Lower motor neurons
III	Myelinated vagus *(ventral vagal complex)*	Social communication, self-soothing and calming, inhibit "arousal"	Nucleus ambiguus
II	Sympathetic-adrenal system	Mobilization (active avoidance)	Spinal cord
I	Unmeyelinated vagus *(dorsal vagal complex)*	Immobilization (death feigning, passive avoidance)	Dorsal motor nucleus of the vagus

Table 9 The Phylogenetic stages of Polyvagal Theory's stress activation responses. [Duplicated from Porges, 2007 (8)]

Functionally, when the environment is perceived as 'safe', the visceral state is regulated in an efficient manner to promote growth and restoration (e.g., visceral homeostasis), but when the environment is

[16] They respond to challenges in a phylogenetically-determined hierarchy consistent with the Jacksonian principle of dissolution. Jackson proposed that in the brain, higher (i.e., phylogenetically newer) neural circuits inhibited lower (i.e., phylogenetically older) neural circuits and "when the higher are suddenly rendered functionless, the lower rise in activity" and describe the sequence of ANS response strategies to challenges. (John Hughlings Jackson (1835-1911) – known as "The Father of English Neurology".)

perceived as ‘threatening’, two more primitive neural circuits to regulate defensive strategies (i.e., fight-flight and freeze behaviours) are retained. Social behaviour-and-communication and visceral homeostasis states are incompatible with the neurophysiological states and behaviours promoted by the two neural circuits that support defence strategies. Thus, via evolution the human nervous system retains all three neural circuits of ANS activation, which are in a phylogenetically organised hierarchy. In this hierarchy of adaptive responses, the newest circuit is used first (III), and if that circuit fails to provide safety the older circuits (II and I) are recruited sequentially.

Bearing the aforementioned in mind, if the ANS responses observed in studies 2 and 3 are considered with the deep breathing visit representing arguably a safe/nurturing environment with a high degree of interpersonal interaction he sham breathing visit with some interaction, as representing a neutral environment, and the stress test that of a threatening/stressful environment, as summarised in table 10, one will then have a continuum of ANS responses of various subject vulnerability phenotypes across progressively increasing environmental stress states.

	SCR	CVT (Ventral Vagal Complex)	CVT (Dorsal Vagal Complex)	Sensitisation Response	Modulation (type & group)	Vulnerability Phenotype	Environment	Subjective Perception
Deep breathing	↑	↑↑		De-Sensitised	Deep Breathing (Sensitiser)	Vulnerable	(+)	Safe
	↑	↑↑		De-Sensitised	Deep Breathing (Non-sensitiser)	Resistant	(+)	Safe
Sham breathing	↑↑	↓		Sensitised	Sham Breathing (Sensitiser)	Vulnerable	(+/-)	Neutral
	↑↑	↑		Not-Sensitised	Sham Breathing (Non-sensitiser)	Resistant	(+/-)	Neutral
Stress test	↑		↑	Sensitised	Stress Test (Sensitiser)	Vulnerable-Resistant	(-)	Threatening
	↑↑		↓	Not-Sensitised	Stress Test (Non-sensitiser)	Resistant-Resistant	(-)	Threatening

Table 10 *An 'Executive schematic summery' of the results for study 2&3.*

Using the subject group's sensitisation status the ANS responses can now be contrasted and better understood with the ANS activation phase as proposed by Porges *et al.* across different stress environments. In Figure 95 it will become clear how the sensitisers PNS (ΔCVT) activation represents a U-shape, where the vagal is both adaptive, and maladaptive, depending on the specific lower vagal motor neuron activated, which then leads to subsequent sensitisation in phases II and I, and desensitisation during phase III. The non-sensitisers on the other hand demonstrate an S-shaped curve, which does not lead to hypersensitisation in any of the phases. During the deep breathing visit, both groups demonstrate similar increases in CVT, consistent with phase III's NA activation (the "mammalian vagus"). It is during the sham-breathing visit that differences in the groups become clearer, with the sensitisers demonstrating a greater degree of vagal withdrawal compared to the non-sensitisers, consistent with phase II. Similarly during

phase I, the subjects that did not sensitise had lower activation than those that sensitised, consistent with phase I's DMNX activation (the "vegetative vagus").

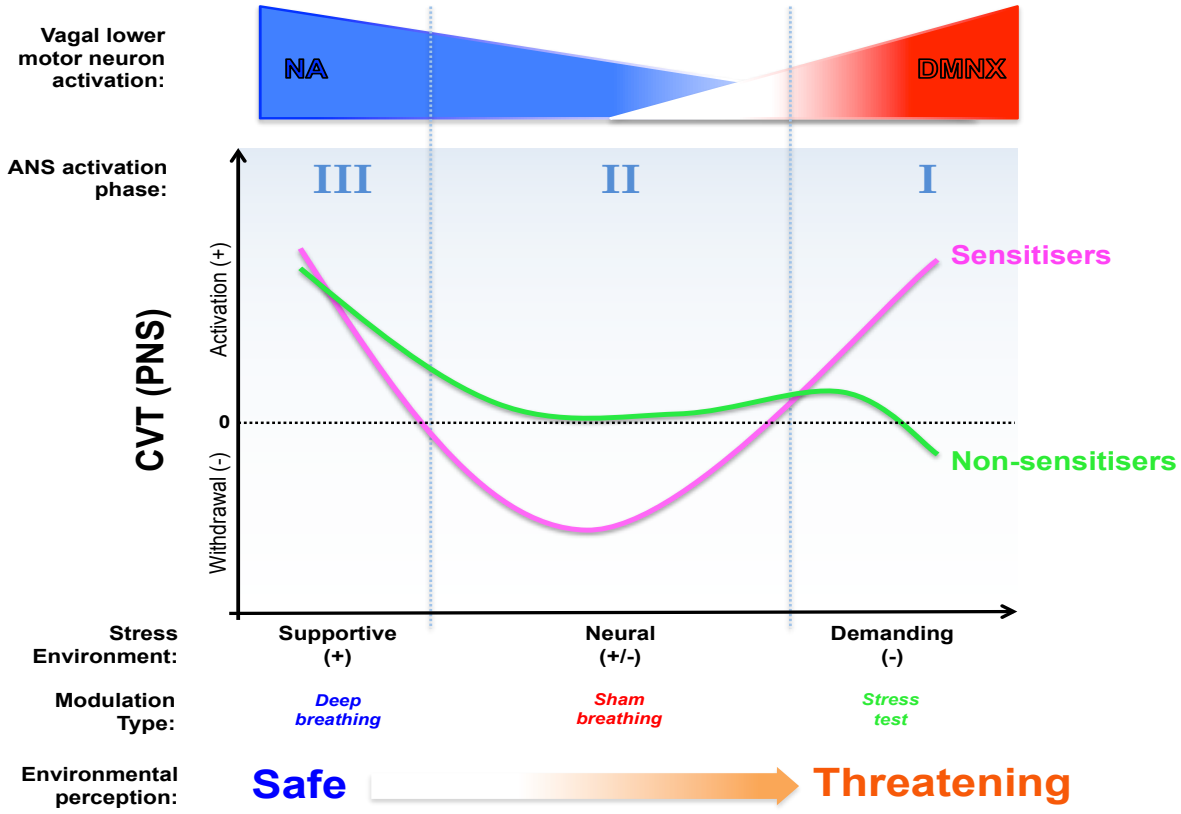


Figure 95 Illustrates the changes in Cardiac Vagal Tone (CVT) of the Parasympathetic nervous system (PNS) across three different environmental stress conditions ranging from 'supportive/safe' (left) as experienced during the deep breathing-experimental modulation procedure, threw 'neutral' (middle) as experienced during the sham breathing-experimental modulation procedure, to 'demanding/threatening' (right) as experienced during the stress test-experimental modulation procedure. This gives rise to three distinct different activation patterns as described by S. Porges (100) and illustrated by the roman numerals: III, II & I, coinciding with different vagal lower motor neuron activation, illustrated above as ranging from left, mainly Nucleus Ambiguus (NA) to the Dorsal motor neuron nucleus of the vagus (DMNX) on the right. In the foreground is a schematic representation of the changes in stress responses as observed during studies 2 & 3, for the sensitisers (pink graph), and the non-sensitisers (green graph) to acid infusion induced oesophageal pain hypersensitivity (OPH).

Similarly in Figure 96 the differences in SNS (ΔSCR) are illustrated during the same stress activation phases as in table 9 for the differing subject sensitisation status groups. Once again during phase III (deep breathing visit), both groups demonstrated low SNS activation/withdrawal. During

phase II (sham breathing visit) there is SNS activation to a degree where the between group differences are noted, but not statistically significant. It is during phase I that a clear difference can be detected, with the non-sensitisers able to mount an appropriate sympathetic response, compared to much lower activation by the subject group that sensitised (stress test).

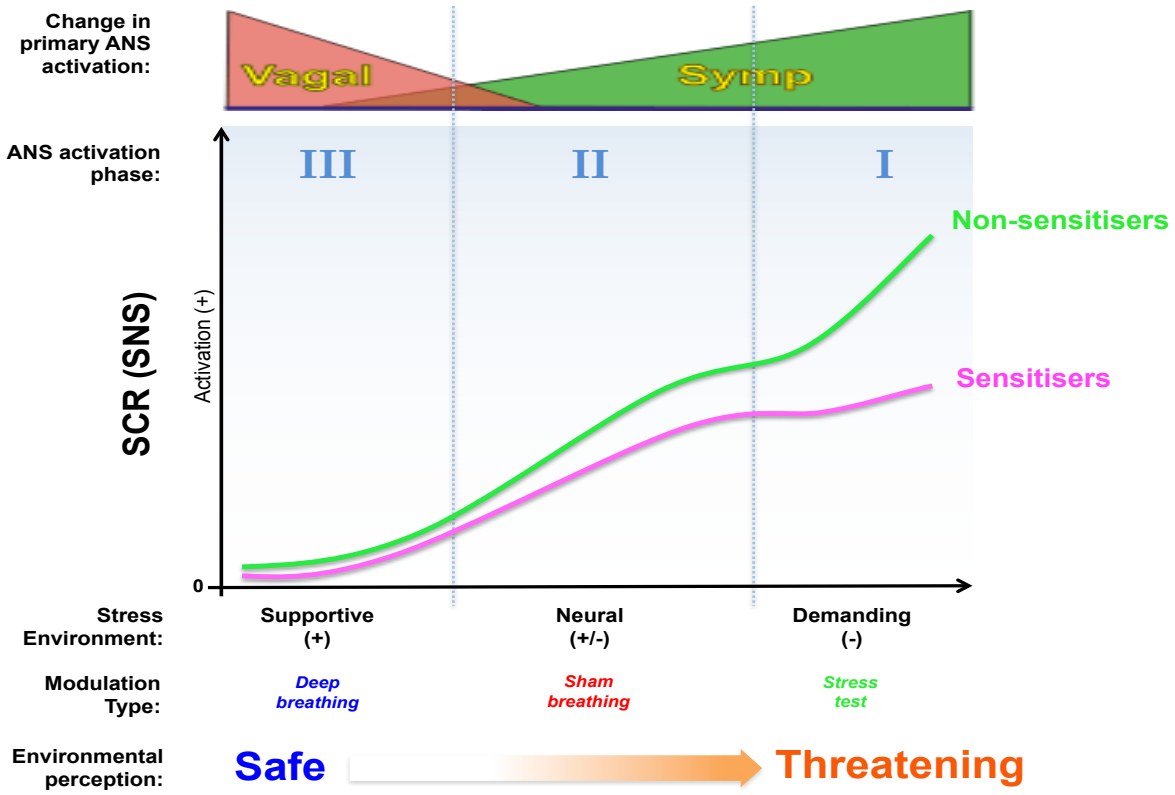


Figure 96 Illustrates the changes in sudomotor activation of the Skin Conduction Response (SCR) under control of the Sympathetic nervous system (SNS) across three different environmental stress conditions ranging from 'supportive/safe' (left) as experienced during the deep breathing-experimental modulation procedure, threw 'neutral' (middle) as experienced during the sham breathing-experimental modulation procedure, to 'demanding/threatening' (right) as experienced during the stress test-experimental modulation procedure. This gives rise to three distinct different activation patterns as described by S. Porges (100) and illustrated by the roman numerals: III, II & I, coinciding with change in primary autonomic nervous system (ANS) activation, illustrated above as ranging from left, mainly Vagal (also known as the 'vagal brake' to unimpeded sympathetic activation on the right. In the foreground is a schematic representation of the changes in stress responses as observed during studies 2 & 3, for the sensitisers (pink graph), and the non-sensitisers (green graph) to acid infusion induced oesophageal pain hypersensitivity (OPH).

It is with the incorporation of attachment theory that the psychological underpinnings of the above observed ANS responses potentially become clearer and possibly more understandable.

In the extreme, the true freeze response (phase I) is dangerous to mammals as the high tone immobility response of the DMNX vagal system is lethal in mammals. Whereas high tone from the NA-vagal system (phase III) may be beneficial in adaptive significance of mammalian affective processes including courting, sexual arousal, copulation, and the establishment of enduring social bonds (attachment). In the development of enduring pair-bonds the mammalian vagus communicates safety and trust, via oxytocin and vasopressin, between the hypothalamus and the medullary source nuclei of the viscera vagus. (323)

The higher cognitive processes of the prefrontal cortex calm the stress response and establish effective social connections by using facial muscles, establishing eye contact, modulating tone of voice and listening to others (attachment behaviour). This increases the influence of the myelinated vagus, which is calming and decreases the stress response and is metabolically more efficient. (324)

The vagus is asymmetrical with the left and right sides performing different tasks, with the right vagus most active in cardiovascular regulation. Primary emotions are related to autonomic functioning since they are often survival related, and hence they must be integrated into cardiopulmonary regulation. Emotions have a right limbic bias, as does the brainstem medullary structures controlling visceral function. Only when the environment is perceived as "safe" is there cortical regulation

of the visceral pathways, because while under threat, cortical control of brainstem structures would compromise the individual's ability to mobilise (phase II). Therefore when stressed or in danger, cortical control of brainstem is "inhibited" and the brainstem structures are "disinhibited" to allow the SNS to efficiently increase metabolic output (phase I).

Vagal stimulation releases noradrenaline into the amygdala strengthening memory storage and regulates arousal, memory and affective responses to emotionally laden stimuli. This is the mechanism by which peripheral adrenaline released during the fight-flight response, activates noradrenaline release in the limbic system strengthening memory of certain events. Since adrenalin cannot cross the blood brain barrier it activates the vagus nerve, which in turn stimulates neurons in the NTS. Visceral organ vagal afferents from the head, neck, thorax, and abdomen relay information to the NTS, that in turn release noradrenaline into the memory processing areas of the amygdala and hippocampus. (325) This activates long-term memory storage of emotionally laden events. It is these long term stored limbic memories of "emotionally laden events", that give rise to one's inner-concept of a "secure-base", that forms the discriminating factor in the observed inter-individual variation of the degree of cortical control of brainstem structures controlling the individual's ability to mount an appropriate mobilisation response. Therefore when stressed or in danger, the inhibitory cortical control of the brainstem is modulated by the limbic memory storage and brainstem structures, that then affect the degree of SNS "disinhibition" that is allowed to increase metabolic output in response to an environmental stress stimuli (phase I).

Craig *et al.* (326) explains how emotions arise from feelings in our organs and gut. The feelings are sent via the vagus nerve to the Anterior Insular

Cortex (AIC) in the brain. The AIC captures feelings over time and stores them as snapshots of feelings. This is our "working emotional memory". These feelings are massaged and integrated with the social exchange to give us both an emotional response to the world around us as well as a safety-driven response strategy (e.g. an adult attachment style). Almost any activity will involve the combined interaction of the various safety strategies. The bottom line of which is that one is constantly adjusting to meet the challenges posed by the world. The results of studies 2 and 3 give one a look at how this potentially works.

Safety, as an inner sensation, not a mind-based concept, is the feeling of "inner-security" that Bowlby and Ainsworth *et al.* called one's "secure-base," and one's "fundamental need of attachment to others, for healthy physical and emotional/mental, functioning." (119, 327, 328) Attachment theory describes the dynamics of long-term relationships between humans. Its most important tenet is that an infant needs to develop a relationship with at least one primary caregiver for social and emotional development to occur normally. It explains how much the parents' relationship with the child influences development. Attachment theory is an interdisciplinary study encompassing the fields of psychological, evolutionary, and ethological theory. Ainsworth *et al.* (329) introduced and reinforced the basic concept of the "secure base" and developed a theory of a number of attachment patterns in infants: secure attachment, avoidant attachment and anxious attachment, that later was expanded to include a fourth type,[17] and applied it to adults.

"Feeling safe within" and "having a secure-base", is now increasingly understood as being as vital to one's physical, emotional and mental

[17] The Disorganised attachment.

health, as oxygen is for one's on-going survival. It is known that feeling unsafe within or not having a secure-base 'autonomically' triggers our ancient freeze/flight/fight defences, through the above-mentioned process of "neuroception", and thus produces these individuals more vulnerable. It has been previously reported that when one does not feel 'safe within', or has no 'secure-sense-of self', the resulting tension is then observable in the changes seen in visceral responses. Depressing this neural system has several behavioural consequences including flat affect, aprosody, difficulty in phoneme recognition, articulation problems, auditory hypersensitivity, and behavioural state regulation issues. (330) Although these symptoms are nonspecific regarding differential psychiatric or behavioural diagnosis, they are shared by many children with developmental disorders.

With the above-mentioned in mind, when one considers the 'vulnerability phenotype' column of table 10, the most vulnerable subjects (subjects sensitising at sham breathing) had a 40% vulnerability score, as opposed to the 'resistant phenotype'-groups (subjects who did not sensitise at sham breathing) 33%, on VASQ. Of the resistant phenotype group, those subjects who sensitised under stress had a 50% VASQ vulnerability. The tentative results with regard to the adult attachment style indicate that it was predictive of the sensitisation response status. This finding could represent a replication of that made by Meredith *et al.* (22) and Davies *et al.* (331) working in similar fields of chronic pain disorders, and can aid in a better understanding of the context of underlining ANS effects in analgesia.

The main weakness of this part of the study was the small sample size (type II error), and that attachment was assessed using a brief, self-report measure. The assessment of attachment therefore reflects individuals'

subjective perceptions of their close relationships, which may be vulnerable to reporting bias. Whilst other more comprehensive assessments of attachment, delivered by self-rated questionnaire or interview, were available, these were considered too lengthy for inclusion in the laboratory setting of this study.

Closely related to attachment is the significance of alexithymia in affording a degree of vulnerability to individuals in study 3. If a subject is able to 'understand', or 'make sense' of their inner emotional and/or visceral – states, i.e. neuroception, the individual is able to 'cortically' mediate the resulting ANS response more effectively, as described above, and in so doing maintain a higher visceral PT. Nyklícek *et al.* (298) looking at 41 healthy volunteers, found that alexithymia was associated with low tolerance to experimental pain stimuli, a finding that Ahlberg *et al.* (297) replicated looking at 750 subjects in connection with temporomandibular pain disorder. Dealing with alexithymia and attachment issues has now become the object of a major field of research known as "interpersonal neurobiology", where Siegel *et al.* are developing novel therapeutic interventions dealing with these complicated interdisciplinary patients. A concept known as "mindsight" has been coined to address alexithymia and disregulation therapeutically. (332)

Finally, with regard to study 3 there remains the issue around anxiety. Here the observed contradiction where the non-sensitising subjects had a higher state anxiety when compared to those who sensitised previously, goes against findings in study 1 and heretofore experiences using this model by Sharma *et al.* (311) This is however not without precedent, as Thibodeau *et al.* (296) found looking at 95 nonclinical participants (55% women) that anxiety sensitivity was associated with an

increased pain tolerance, "*a novel finding needing further examination*." (296) Here pain was induced by using heat and cold stimuli, administered by a Medoc Pathway Pain and Sensory Evaluation System. This arguably represents a stressful experience in healthy volunteers (possibly a resistant group), and it would be interesting to see if the results remained consistent when repeated in patients (a vulnerable group). With the understanding gleamed from the 'phase I protective' SNS activation (figure 96) a possible reinterpretation consistent with the present findings would suggest that anxiety in this context represents an appropriate 'adaptive' response from a more resistant phenotype, and just like the PNS could represent a 'double edged sword', where, depending on the context. Activation can be both 'protective' and 'harmful'. This hypothesis is strengthened by the observation that the non-sensitising group's trait anxiety was lower than that of the sensitising group. The finding would be consistent with a more resistant sub-group. Further, Holtmann *et al.* (333) found that when acute psychological stress was induced in 14 healthy subjects and compared with endogenously stimulated gastric acid output, there was a great individual variability in gastric acid response to acute mental stress, and that this variability may be "*attributed to differences in personality traits.*" (333) They go on to describe inter individual differences in blood pressure and heart rate responses, suggesting personality (in their case, impulsivity) mediated differences in cortical control of ANS responses, as described above, effecting gastric acid secretion.

Hence the application and reinterpretation of previously contradicting findings could possibly become more understandable and clinically applicable. This would suggest that for clinicians to reach greater clinical efficacy in future, they would have to have a working knowledge of a patient's vulnerability phenotype, in order to best match to most

effective treatment regimen. Characterisation would also allow appropriate individualisation of management to the underlying mechanism. The results from studies 2 and 3 for the first time give us an explanation of underlying neural mechanisms for the observed spectrum in phenotype vulnerability, but further study is necessary to clarify this.

4.6 Conclusions

In conclusion, studies 2 and 3's findings represent the first human study addressing the pivotal role of the ANS in mediating visceral pain hypersensitivity as induced in the proximal oesophagus by acid infusion in the distal oesophagus. Study 2 provides evidence for how sensitisation can be prevented by deep breathing through its action on the increasing PNS tone, and study 3 demonstrates the paradoxes with regard to ANS regulation across a continuum of environmental stress levels. It also highlights the need for a deeper understanding of the vulnerability phenotypes involved.

Studies 2's results represent a novel human intervention study addressing the key role of the PNS in mediating visceral pain hypersensitivity. It has now been shown that the induction of acid-induced hypersensitivity is altered by physiological influencing PNS tone. This finding strongly indicates that the PNS plays a central role in the development of central sensitisation. Further study is now needed to investigate the potential of therapeutically manipulating PNS tone in the management of visceral pain.

It is now important to study the effect of co-administered atropine and deep breathing on sensitisation. This could potentially examine and contrast the contribution and importance of the neurobiological

pathways that underlie deep breathing induced PNS analgesia. The degree of associated distraction that accompanies all paced breathing techniques could be examined and potentially highlight their therapeutic difference and impact. Distraction is a manifold phenomenon; the analgesia that was observed in study 2 could plausibly be due to the observed reduction in anxiety. The results should also be validated by means of an unrelated cohort in another study centre.

Studies 3's results provide evidence that explain and clarify the hitherto poorly understood multifactorial ANS regulatory mechanisms of visceral pain hypersensitivity in non-sensitising subjects. This group has never been studied using this model before, and it is with the inclusion of their ANS response to acid that allows us for the first time to observe and compare the ANS response of healthy subjects across the complete spectrum of visceral hypersensitivity reactions as induced by this model.

With the simultaneous examination of all three parts of the biopsychosocial triumvirate, an important synthesis could be made between developmental psychology, neurobiology and gastroenterology. This allows us to reinterpret previously conflicting results with more clarity and potentially greater therapeutic advantages. With the incorporation of attachment and polyvagal theory, study 3's results demonstrate the paradoxes surrounding ANS regulation with regard to central sensitisation across a continuum of environmental stressors. It also highlights the need for a deeper understanding of the vulnerability phenotypes involved, and further study is now warranted to clearly define the psychological, physiological and genetic markers of the vulnerability phenotypes. Future investigation is also needed to examine the potential of therapeutically manipulating ANS tone (e.g.

psychologically / pharmacologically) in the management of chronic visceral pain syndromes.

5 Effect of Psychopharmacological Modulation with Atropine on Acid Induced Oesophageal Hypersensitivity - Study 4 *(Atropine challenge pilot study)*

5.1 Introduction

Visceral pain is a complex phenomenon with sensory-discriminative, affective-motivational and cognitive-evaluative components. (334) In study 2 it was demonstrated that during deep breathing there is an increase in CVT such that oesophageal acid infusion failed to cause sensitisation. This observation supports the hypothesis that withdrawal of parasympathetic tone is associated with sensitisation whereas an increase is protective and reduces sensitisation. This data supports the notion that the parasympathetic nervous system may have anti-hyperalgesic properties in the human viscera, and that anxiety may predispose to greater post-injury gut sensitisation through the withdrawal of vagal tone. Conversely, it has been demonstrated that increasing CVT through deep breathing reduces sensitisation in the viscera. However, the exact mechanism of how this decrease in sensitisation occurs is not clear despite evidence pointing to the 'up' modulation of the parasympathetic nervous system as the likely cause for the anti-hyperalgesia.

It is important to study the effect of co-administered atropine and deep breathing on sensitisation. If potential amelioration of the degree and development of visceral hyperalgesia is due to deep breathing induced PNS, this should be negated with pharmacologically reduced PNS tone by an anti-cholinergic. This could potentially examine and contrast the contribution and importance of the neurobiological pathways that underlie deep breathing induced PNS analgesia. The degree of

associated distraction that accompanies all paced breathing techniques as in study 2, should be clarified. Distraction is a manifold phenomenon; the analgesia that was observed in study 2 could plausibly be due to the observed reduction in anxiety. Study 2's results should also be validated by means of an unrelated cohort in another study centre.

The proposed study aims to clarify this phenomenon, as well as elaborate on the conditions of desensitisation with the addition of a means of PNS anti-cholinergic blocking with atropine sulphate, administered intravenously. Atropine is used, as it is an established form of blocking vagal tone in similar experimental studies. The resulting re-sensitisation of volunteers that previously desensitised with the deep breathing modulation protocol during oesophageal acidification will be conclusive in establishing the role of the PNS in deep breathing induced desensitisation.

It is thus hypothesised that the physiological deep breathing induced PNS desensitisation will be inhibited with the anticholinergic atropine, causing a re-sensitisation in oesophageal pain hypersensitivity through the unopposed effect of the sympathetic nervous system.

5.2 Materials and Methods

5.2.1 Ethics Committee Approval

All protocols for this study were submitted and approved by the Research Ethics Committee of North Jutland, Denmark (ref: N-20120065vII). See section 2.1 (page 77).

5.2.1 Subjects

32 healthy asymptomatic adult male and female volunteers, aged 18 to 50, were recruited by advertisement. Screening for acceptability for inclusion and exclusion criteria was completed as described in section 2.2 (page 77).

5.2.2 Oesophageal Manometry

For this study standardised oesophageal manometry (183) was performed in the first five subjects of study 1 to determine the positions of the upper and lower oesophageal sphincter (UOS and LOS) from the nostril. As the LOS positions on these first five subjects were found to be accurate enough for the purpose of this study, only the 'pH change' pull back technique as described in section 2.3 (page 78), was used for the remaining 15 subjects.

5.2.3 Psychological Assessment

For study 4, only Spielberger – Trait and State Anxiety Inventory STAI was used. (section 2.11, page 85) The Trait questionnaire was completed during the screening visit, wiles the State questionnaire, was completed at the start and end of visit 2 (V1) and visit 3 (v2), as study 4's endpoint analysis did not require more extensive psychological examination.

5.2.4 Other Methods of Measurement

All other methods of measurement; Catheter Assembly (section 2.4, page 78), Oesophageal acid infusion (section 2.4, page 78), Oesophageal pH monitoring (section 2.6, page 80), Pain Threshold Measurements (section 2.8, page 82). Measurement of the Autonomic

Nervous System (section 2.1, page 86) and Respiratory Monitoring (section 2.16, page 99) was performed as described in their specific sections, except that the skin conductance response could not be measured, as required equipment was not available in the Danish research site. Screening visit protocol was followed for the first visit, and all non-sensitisers were excluded. For the following two visits the exact same protocol was followed as for the deep breathing modulation, used in study 2, with the exception of the administering of placebo (0.9% normal saline solution), or atropine, as described in chapter 2 (section 2.20.4, page 115) and illustrated in figure 97 below.

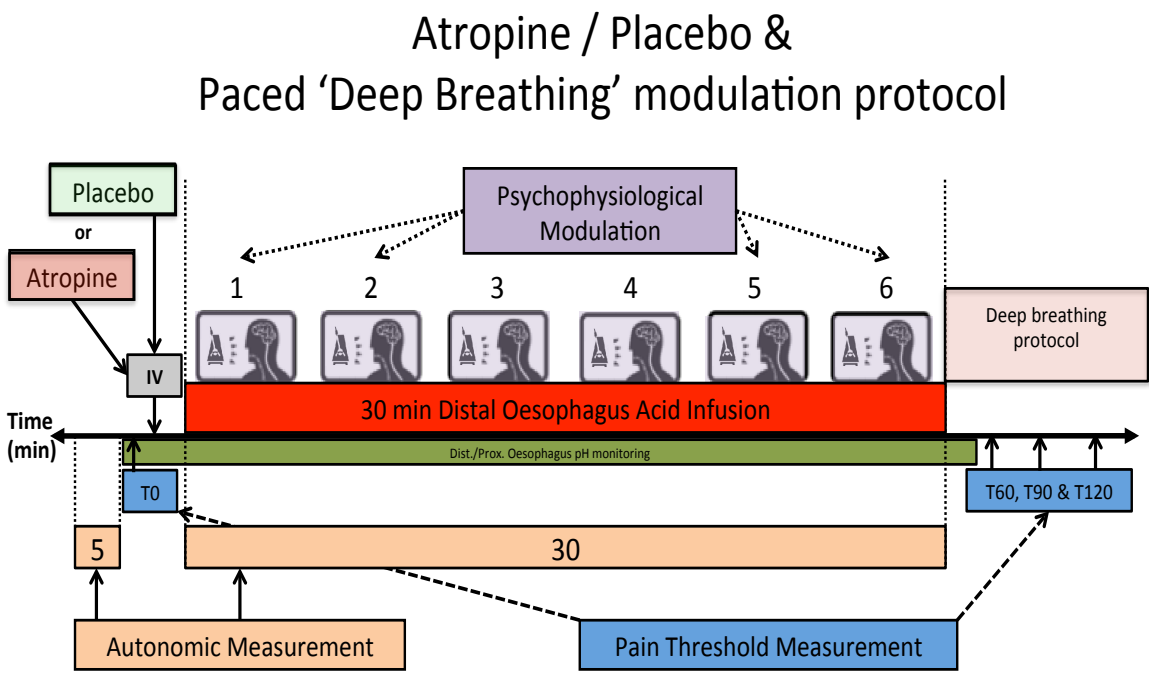


Figure 97 *Diagram illustrating the psychophysiological modulation protocol for the atropine-placebo study. The subject was paced to perform 6 deep breaths on six occasions (purple figures) during the 30minitus acid infusion period (red bar) on all visits. Atropine or placebo was administered 5mins before the start of acid infusion. Autonomic measurement (brown bars) was done before and during the acid infusion. Pain thresholds (blue bars) were done before and three times after acid infusion. PH-metry (green bar) was started 20mins before acid infusion, and stopped 30mins after acid infusion ended (see figure 41).*

5.2.5 Study Procedure, Experimental Design & Protocol

The experimental study design was that of a prospective randomised two-tiered double-blinded longitudinal crossover cohort study. (Figure 98) The study procedure was followed as described in section 2.17 (page 99), i.e. using the 'three research assistants' method. The experimental protocol was used as described in section 2.18 (page 101), with 'time and events' proceeding as outlined in figure 41 (page 102), with amendments as mentioned in section 5.2.3.

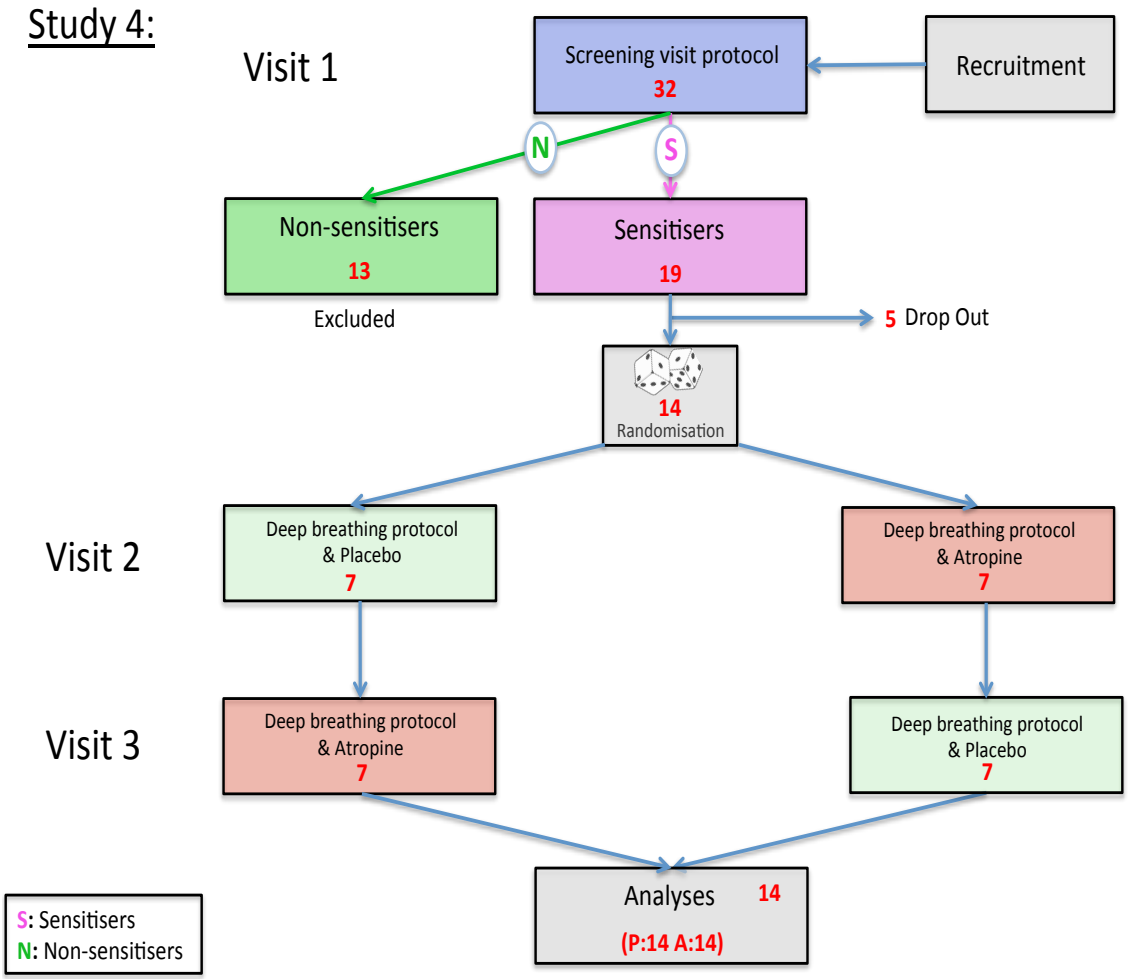


Figure 98 *Flow diagram illustrating the final numeric outcome of participants in study 4. The experimental study design was that of a prospective randomised two-tiered double-blinded longitudinal crossover cohort study. Sv: Screening visit, P: Deep breathing & placebo, A: Deep Breathing & atropine.*

5.2.6 Data Handling, sample size and Analysis

Demographic, pain threshold and autonomic data were normally distributed hence data are presented as mean ± SD, with parametric analysis. The variability was computed for the main effects of each subject's change in PT over time points (ΔPT & time). All statistical analysis was completed as described in section 2.21 (page 120).

5.3 Results

During acid infusion, pH fell to <2.0 in the distal oesophagus of all subjects but remained >6.0 in the proximal (unexposed) oesophagus. The most common symptom reported with acid infusion was nausea. Other sensations included a cold sensation in the chest region, feeling of hunger and / or heartburn.

5.3.1 Demographic Data:

A total of 32 healthy volunteers were recruited and assessed for criteria eligibility. The healthy volunteers were recruited through an already established database at the Aalborg University Hospital, Denmark. All had normal medical assessments comprising of medical and surgical history with physical examination, heart rate (HR) and blood pressure (BP) recording, baseline electrocardiograph (ECG) and routine haematological and biochemical laboratory tests. The age range was from 21-49 years with a mean age of 28 ±9.11years. There were no obese or underweight subjects and the average body mass index (BMI) was 23.10 ±2.75kg/m^2. All subjects were recruited from a European (Danish) ethnic backgrounds All subjects were acid infusion naïve, with 59%

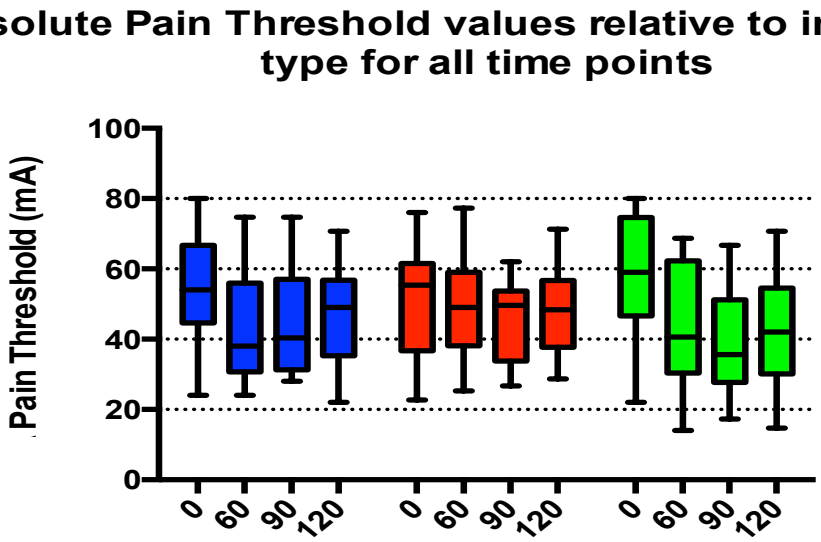
sensitising to acid infusion, allowing 19 subjects to be approached for phase two of the study. Three subjects withdrew from the study due to logistical limitations, and two withdrew consent following their experience during the screening visit.

14 Subjects were randomised into two groups for their second visits. To randomise subjects without bias, www.randomisation.com (an approved statistical randomisation software package) was used. Subjects were randomised in groups of n=5. For the final analysis 14 subjects (6 male) were included. Due to technical problems with equipment, only 13 subjects' autonomic data could be analysed. (Figure 68)

5.3.2 Pain Tolerance Threshold Data of Proximal Oesophagus and Foot

Absolute threshold data for the proximal oesophagus at (T0) and after acid infusions (T60, T90, T120) are shown in Figure 99(A) & table 11(B) below.

A:



B:

	Screen-ing Visit				Deep breathing + Placebo				Deep Breathing + Atropine			
Time point	0	60	90	120	0	60	90	120	0	60	90	120
Mean	**58.05**	**43.62**	**39.10**	**41.43**	**52.38**	**48.38**	**46.29**	**47.95**	**54.38**	**43.10**	**44.76**	**47.05**
Std. Deviation	19.11	18.20	14.73	15.70	15.23	13.95	10.98	12.09	15.74	15.00	14.28	14.75

Table 11

Figure 99 (A&B) Absolute values for proximal oesophageal pain thresholds before (T0) and after (T60 T90 and T120) acid infusion with (blue) Screening visit, (red) Deep breathing & placebo and (green) Deep breathing & atropine (n = 14)

The mean individual 'pre/post-acid infusion' changes in pain threshold (ΔPT) for all subjects in the proximal oesophageal, during screening visit, deep breathing and placebo, and deep breathing and atropine with the mean group value (SD) for each time point, are shown in Figure 100(A, B and C). Deep breathing and placebo significantly reduced the development of acid-induced hypersensitivity in the proximal oesophagus compared to screening visit. With deep breathing and atropine there was a greater degree of acid-induced hypersensitivity, but not to the degree observed during screening visit.

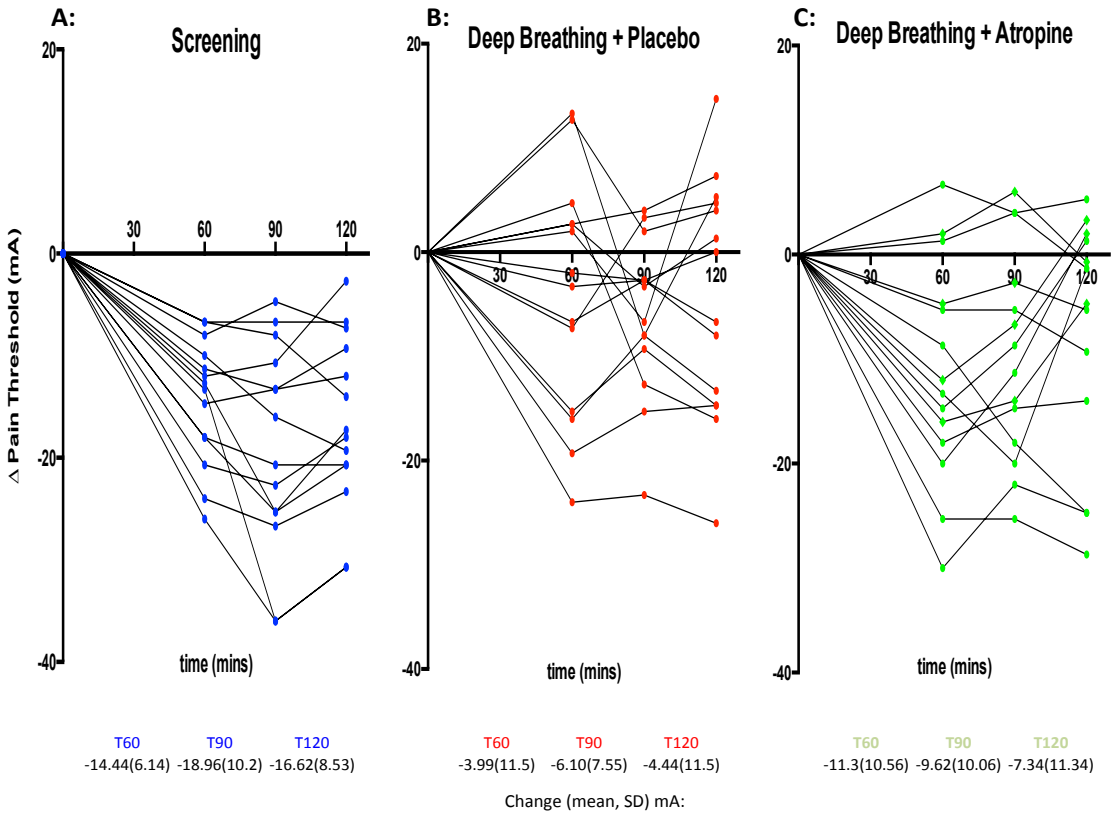


Figure 100 ***Individual values of change for proximal oesophageal pain thresholds (ΔPT) for time points T60, T90 and T120, following acid infusion with (A) screening visit, (B) deep breathing & placebo, and (C) deep breathing & atropine.***

Two-way MANOVA analyses, comparing the influence of effect for 'Deep breathing and placebo' and 'Deep breathing and atropine' vs. screening visit modulation's mean ΔPT for the proximal oesophagus with that of modulation type, across all time points. A strong statistical significance with regard to deep breathing and placebo, for 'interaction' ($p=0.01$) and 'time points', contributing 18.33% at $p<0.0001$, was observed. (Red graph, Figure 101(A)) Regarding deep breathing and atropine, there was not a statistical difference for 'interaction' ($p=0.215$), but significance was achieved to a lesser degree across 'time points', contributing 3.91% at $p<0.0119$. (Green graph, Figure 101(A))

In the comparison of the pre and post acid differences in average means of pain threshold (Δ Avr PT) for the proximal oesophagus between the screening visit and the two modulations, a statistical difference was found with regard to screening visit vs. deep breathing and placebo, where the between group difference was Δ11.8 ±13.03mA, p=0.0048. For screening visit vs. deep breathing and atropine there was not a statistical between group difference, Δ7.24 ±13.06mA, p=0.058. The between modulation group difference was not significant, Δ-4.6 ±9.50mA, p=0.094, using two-tailed paired t-testing. (Figure 101(B))

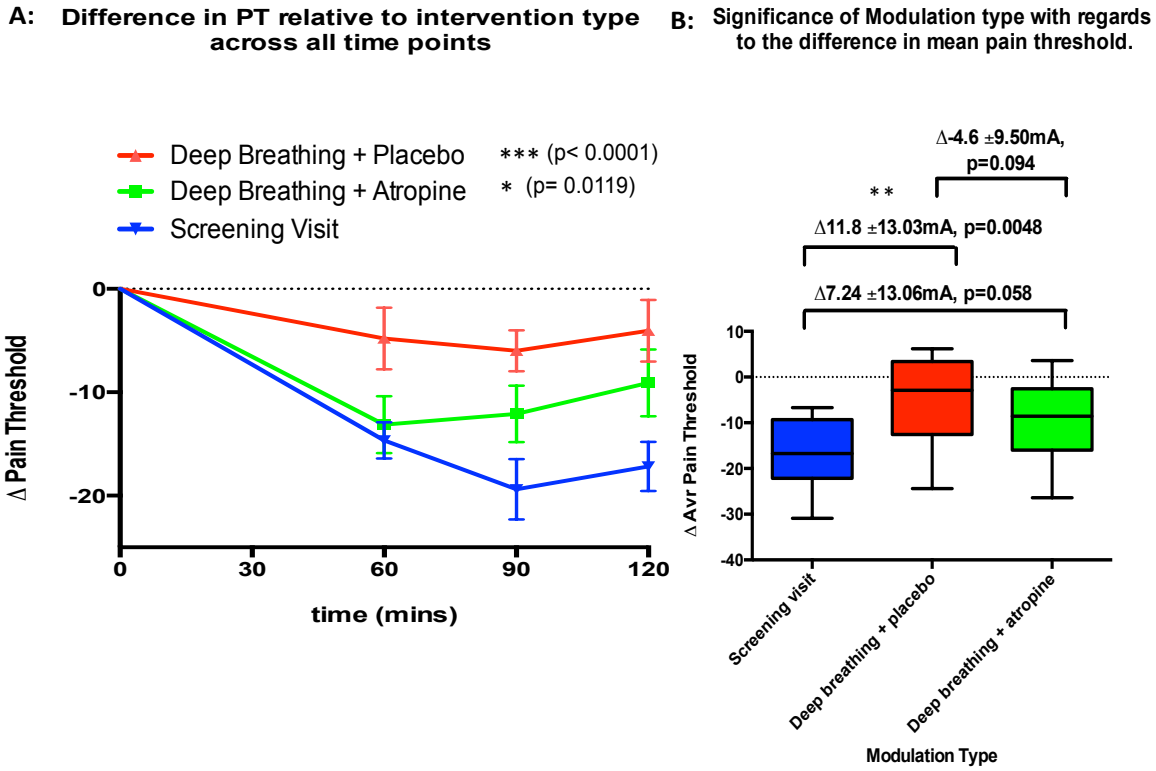


Figure 101 ***A: shows the difference in mean pain threshold (ΔPT) in mA, for the proximal oesophagus between baseline and the three-time points (minutes) after acid infusion, for the different modulation types. B: Shows the difference in average means of pain threshold (Δ Avr PT) in mA, for the proximal oesophagus between pre & post acid infusion, for the different modulation types.***

The foot pain threshold data showed that for all modulations protocols there was no significant change with regard to the screening visit, across

all time points and modulation type; as well as with regard to means comparison analysis.

5.3.3 Autonomic Data

The 'pre/during-acid' infusion change in ANS for deep breathing and placebo protocol served as the 'baseline' to which deep breathing and atropine's ANS changes were compared, and are illustrated below in figure 102(A) & table 12(B).

A:

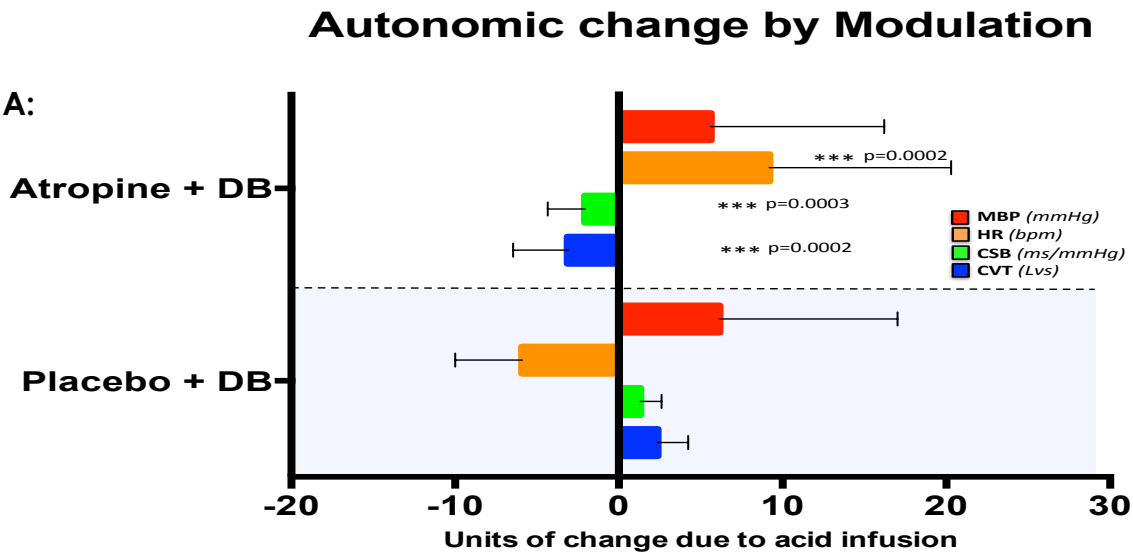


Table 12

B:

Modulation Protocol	ANS Measure	Δ Avr	SD	Difference between means	P value
Atropine + Deep Breathing	MBP (mmHg)	6.7	13.55	-0.71 ± 2.19	0.7871
	HR (bpm)	9.19	11.09	14.82 ± 7.08	0.0002
	CSB (ms/mmHg)	-2.05	2.29	-3.43 ± 0.99	0.0003
	CVT (Lvs)	-3.12	3.33	-5.46 ± 1.54	0.0002
Placebo + Deep Breathing	MBP (mmHg)	7.41	11.36		
	HR (bpm)	-5.63	4.013		
	CSB (ms/mmHg)	1.38	1.298		
	CVT (Lvs)	2.34	1.79		

Figure 102 The comparison between the difference in ANS change between Deep breathing & Placebo (A) and Deep breathing & Atropine (B). In the tables below (A&B) are the mean values of changes (SD & SEM), along with each measure's units and n numbers. Table B, includes the change in means between modulation types with there p value significance.

The changes observed for Deep breathing and placebo protocol demonstrated a post-acid decrease in SNS activation, with coinciding PNS activation, consistent with the observations in study 2. (Chapter 4, Figure 74 (un-shaded graph), page 171) The PNS is hence iatrogenically 'induced' by the behavioural modulation, and unimpeded by the placebo. (Figure 103, shaded graph)

The changes observed during the Deep breathing and atropine protocol demonstrated a post-acid/modulation significant increase in the HR component of the sympathetic outflow, and PNS withdrawal. (Figure 102, un-shaded graph) When compared the difference in SNS was statistically a highly significant decrease in para-sympathetic activation. (Figure 102 and Figure 103) Looking at the ANS comparison, there is a distinct and significant difference in activation between placebo and atropine, where there is a marked deactivation of the PNS, with some SNS activation.

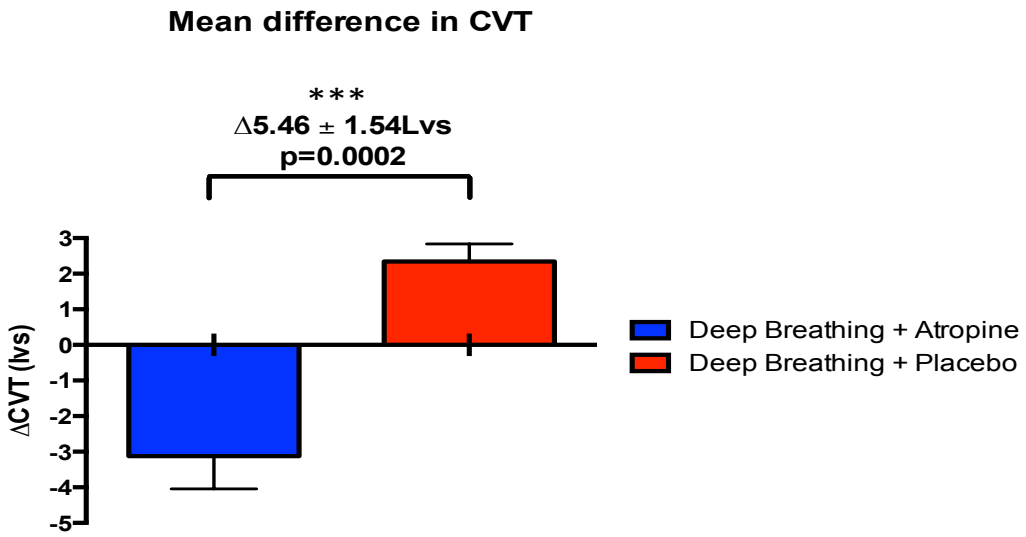


Figure 103 *The comparison of the difference in CVT change between Deep breathing & placebo (Red) and Deep breathing & atropine (Blue).*

5.3.4 Psychological Questionnaire Data

The Danish (study 4) cohort's trait anxiety, 25.79 ±5.90(SD, STAI-T) measured much lower than the British cohort, (study 2: 38.45 ±9.56(SD)) and expectations for similar studies. (267)

Analysis of the State Anxiety Inventory (STAI-S) demonstrated very little variation between visits placebo-or-atropine modulation arm or for pre-and-post modulation measures. This observation combined with the low Trait measure would suggest a psychological robust study group. The self-selection byes that occur with advert recruitment for voluntary invasive experimentation is also a likely contributing factor.

5.4 Summery of Key findings for study 4

5.4.1 Demographic Data:

1. 100% of the subjects were European.
2. All subjects were acid infusion naïve, and 59% sensitised.

5.4.2 Pain Tolerance Thresholds Data:

1. Deep breathing & placebo desensitised significantly at, Δ11.8 ±13.03mA $p=0.0048$, compared to Screening visit, with $p<0.0001$ across all time points. (MANOVA)
2. The distal oesophageal pain threshold data showed Deep breathing & placebo modulation arm caused desensitisation, with regard to the Screening visit, across all time points.

3. Deep breathing and atropine desensitised at, Δ7.24 ±13.06mA p=0.058, compared to Screening visit, with p=0.0119 across all time points. (MANOVA)
4. The desensitisation as a result of Deep breathing and placebo modulation was significantly reduced during the Deep breathing and atropine modulation arm.
5. The foot pain threshold data showed no significant change or difference with regards to visit and observations.

5.4.3 Autonomic Data:

1. Deep breathing and placebo modulation arm demonstrated a post-acid reduction in sympathetic outflow, with para-sympathetic activation consistent with study 2.
2. The changes observed for Deep breathing and placebo protocol were; MBP: 7.41 ±11.36mmHg, HR: -5.63 ±4.013bpm, CSB: 1.38 ±1.298ms/mmHg, and CVT: 2.34 ±1.790Lvs.
3. Deep breathing and atropine modulation arm demonstrated an increase in the HR component of the sympathetic outflow, and PNS withdrawal.
4. The changes observed for Deep breathing and atropine protocol were; MBP: 6.70 ±13.55mmHg, HR: 9.19 ±11.09bpm, CSB: -2.05 ±2.29ms/mmHg, and CVT: -3.12 ±3.33Lvs.
5. Comparison of between arm difference in SNS was, MBP: Δ-0.71 ±2.19mmHg p=0.787, HR: Δ14.82 ±7.08bpm p=0.0002, with a statistically highly significant decrease in para-sympathetic activation of; CSB: Δ-3.43 ±0.99ms/mmHg p=0.0003, and CVT: Δ-5.46 ±1.54Lvs p=0.0002.
6. The between arm comparisons of ANS responses, indicated a marked PNS deactivation, with some SNS activation.

5.4.4 Psychological Questionnaire Data

1. The Danish (study 4) cohort's trait anxiety, 25.79 ±5.90(SD, STAI-T) measured much lower than the British cohort, (study 2: 38.45 ±9.56(SD)) and expectations for similar studies.
2. State Anxiety Inventory (STAI-S) demonstrated very little variation between visits, placebo-or-atropine modulation or for pre-and-post modulation measures.

5.5 Discussion

The results of study 4 provide evidence that visceral pain hypersensitivity, induced in the proximal oesophagus by acid infusion in the distal oesophagus, is prevented by deep breathing through its action on the increasing PNS tone. The analgesic effect of deep breathing is partially reversed by addition of an anti-cholinergic demonstrating that development of oesophageal pain hypersensitivity, through central sensitisation, is influenced by the PNS.

The first phase of study 4 was to exclude non-sensitisers following screening visit. Previous literature quotes that up to 30% of healthy volunteers will not sensitise to acid. (175) In this study using the model of acid induced oesophageal hypersensitivity it has been demonstrated that this result was replicated: such that 19 out of 32 (i.e.: 41%) of healthy volunteers did not sensitise to acid infusion. Enhanced sympathetic dominance to oesophageal acid infusion has been documented in patients with gastro-oesophageal reflux disease. (GORD) (154) It can therefore be speculated that in this model the difference between these two groups lies in their capacity to maintain or withdraw parasympathetic tone during acid infusion at this specific stress response

level. Withdrawal of parasympathetic tone indicates a pro-nociceptive state in the sensitisers, in phase II of the 'stress response', as discussed in chapter 4. On the other hand, the lack of parasympathetic tone withdrawal in non-sensitisers is indicative of an anti-nociceptive state.

Following studies 2 and 3, it is now more understandable why some subjects do not sensitise to acid infusion, as this is due to both peripheral and central factors as previously discussed. Concerning peripheral factors that are implicated in oesophageal hypersensitivity in GORD however, it has been suggested that sub mucosal nerves become exposed to acid through dilated intercellular spaces. Support for this phenomenon has been demonstrated by Sifrim *et al.* (335) whereby they verified that a 30 minute oesophageal acid infusion in healthy volunteers, (in a manner alike to this study), leads to dilated intercellular spaces both at the site of acid infusion in the distal oesophagus as well as in the unexposed proximal oesophagus. Non-sensitisers may therefore have greater resistance to the dilation of intercellular spaces, which does not allow their sub-mucosal nerves to become exposed to the acid. Hence, by sensing less acid, they do not withdraw parasympathetic tone after acid infusion to the same degree as sensitisers. This theory however cannot be confirmed by the present data, as the scope of this research was not to study the mucosal response to acid. What is definite however, is that the subjective response to acid between both sensitisers and non-sensitisers was similar and therefore any differences regarding the exposure of the sub-mucosal nerves to the acid is unlikely to play a significant role in the present study context.

Pain perception can also be influenced by central factors such as certain personality traits and psychological states like anxiety, as previously discussed. Past studies looking into such phenomena using this

experimental model showed that anxiety increases oesophageal hypersensitivity in subjects in the condition of anxiety being artificially induced. (336) Another study also demonstrated that healthy subjects with neurotic and introvert personality traits tended to sensitise more to painful oesophageal stimulation. (271) In the current study however, any significant difference in the levels of anxiety (STAI-trait) and the baseline (T0) levels of pain was not observed. The only significant relationship found between STAI-trait and change in CVT was during placebo and deep breathing (which was then abolished by atropine). The results therefore seem to suggest that psychological factors are not exclusively responsible for the differential response to acid infusion in this cohort.

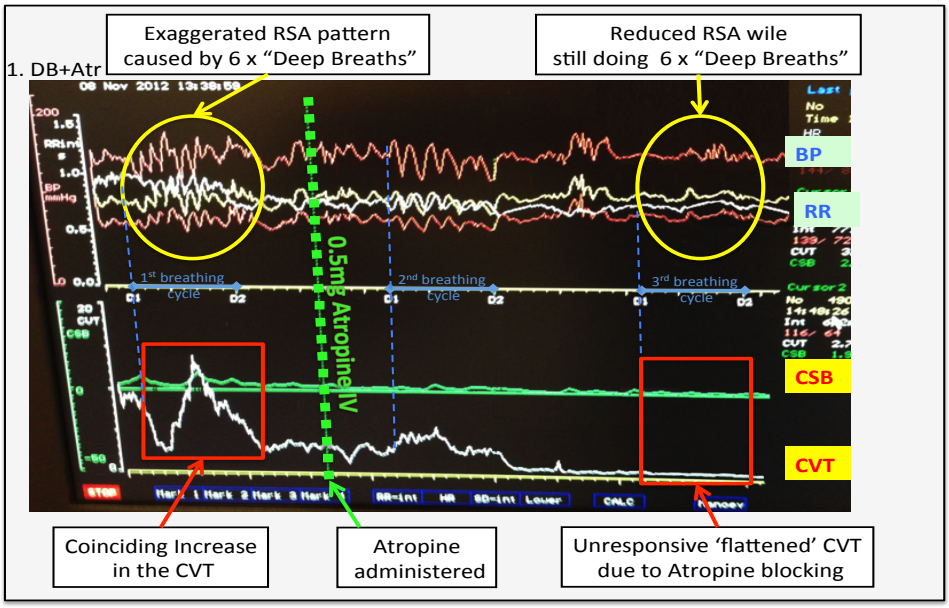


***Figure 104** This diagram shows 3 x deep breathing cycles (numbered in blue) of an healthy volunteer's NeuroScope™ 'screenshot'. The subject received 0.5mg Atropine IV between breath cycle 1 & 2 (green dashed line). The graphs in the upper half of the panel show the blood pressure labelled BP (upper red graph: systolic, lower red graph: diastolic and yellow graph: MAP), and the RR-interval labelled RR (white graph). The graphs in the lower half of the panel shows the CSB (green graph) and CVT (white graph) each labelled as such. The first yellow oval (left) highlights the RSA changes in the BP & RR, brought about by six consecutive breaths of the deep breathing protocol before the administration of the atropine. The red box below highlights the coinciding increase in CSB & CVT from baseline. Compared to this the second yellow oval (right) highlights reduced RSA changes in the BP & RR, indicating that even though the subject was doing six consecutive breaths of the deep breathing protocol the brainstem outflow is now reduced. The RSA, CSB & CVT is noticeably diminished by the second breath cycle, and almost totally unresponsive by the third. The red box on the right highlights the total block of the coinciding CSB & CVT response by atropine.*

Since anxiety is associated to an extent with dysfunction in the autonomic nervous system and the Hypothalamic-Pituitary-Adrenal axis, further study of these systems may provide more objective markers of psychological arousal and distress rather than the questionnaire based scoring tools used in this study, possibly by including the study of subject group's genetic predisposition. In conclusion, the results may explain inter-individual susceptibility to injury-induced sensitisation. Nevertheless, the factors that predict non-sensitisation to acid in this model still remain unclear at present.

The main objective of this study was however to study the anti-hyperalgesic effects of the PNS in modulating the response to sensitisation through acid infusion. Use of the deep breathing protocol was to increase the CVT while use of atropine was used in a sense to 'knock-out' any effects of the deep breathing. The protocol was based on the theory that you can stimulate the vasomotor centre (and NA), which maintains the body's autonomic tone. Physiological stimuli such as paced forced deep inspiration and expiration selectively exaggerates the normal sinus arrhythmia controlled by parasympathetic output of the brain stem vasomotor centre leading to a slowing of the heart rate. This experiment has successfully shown that during deep breathing while on placebo there is indeed an increase in cardio vagal tone and evidence of this can be clearly seen on the Neuroscope™ (Figure 106) while the subject underwent the deep breathing protocol of 6 breaths per minute. Conversely, once atropine was administered, the drug successfully reduced the activity of the parasympathetic nervous system and likewise the cardio vagal tone dropped significantly.

Atropine has a half-life of about two hours, but the data is notwithstanding a degree of circumspection, particularly with respect to

the relatively small sample size, although comparable to other studies of this type. However, it has demonstrated a degree of internal validity in that deep breathing reproducibly increased CVT and alleviated the development of central sensitisation in two unrelated cohorts across two study centres. Additionally, there are inherent limitations to all human pharmacological studies of atropine as its human pharmacodynamics are dose dependent with low dose (c. 2μg/kg IV) and high (>15μg/kg IV) where atropine is considered to be vagotonic and vagolytic respectively. (243) In study 4, largely due to regulatory concerns over cardiovascular safety, a standard dose of 0.5mg of atropine was chosen, which equates to approximately 7μg/kg. Given that only a partial sensitisation was observed in the atropine/deep breathing group, it is possible that the dose that was chosen was vagotonic rather than vagolytic. Therefore it may be possible by increasing the dose of atropine to vagolytic concentrations, i.e. in excess of 15μg/kg, that a complete blockade of the PNS effect of deep breathing may occur thereby allowing re-sensitisation to take place. Ultimately, whether such findings will translate to larger healthy populations and to clinical cohorts, as yet remains uncertain.

Having determined that physiological modulation of the ANS through deep breathing does promote parasympathetic activity, next it had to be resolved whether or not the magnitude of acid-induced oesophageal hyperalgesia could be reduced through promoting activation of the parasympathetic nervous system. The results show that there was a slight fall in thresholds with placebo despite deep breathing. Despite subjects executing the deep breathing protocol the PT did not rise to baseline levels even after 90min post acid infusion. Secondly, when atropine was administered to block the rise in CVT, the sensitisation was significantly more pronounced with a greater drop in PT compared

to placebo intervention. This observation supports the hypothesis that parasympathetic tone withdrawal plays a key role, and is associated with sensitisation while an increase in parasympathetic tone is protective and reduces sensitisation at this phase of the stress response.

To further verify the effect of deep breathing on the PT the same subjects' data from visit 2 and 3 was compared with the data obtained at screening visit. It was found that subjects sensitised the most at screening visit where they didn't perform any deep breathing. There was a statistically significant difference between the average PT of screening visit and placebo and deep breathing groups which can allow us to conclude that in the context of this model by increasing the parasympathetic autonomic nerve tone, oesophageal pain hypersensitivity could be reduced. Of note is the fact that when comparing the difference between the change in PT between screening visit and placebo and atropine groups across all time points, the fall in PT was smaller for the atropine group. Although this difference is not statistically significant on ANOVA analysis, potential explanations for why this occurred could be offered. Deep breathing may also exert its anti-hyperalgesic effect through distracting subjects from experiencing pain. A theory by McCaul and Malott (337) states that "*an individual must attend to a painful stimulus in order for it to be perceived as painful*". Therefore, when subjects are distracted, their perception of pain will also be decreased.

The methodology used in this study was based on a validated model of acid infusion developed by Sarkar *et al.* (311) However, the model has limitations, as it does not fully replicate pathophysiology of GORD. This model uses hydrochloric acid to simulate heartburn/reflux, but clinically

there are other components, such as enzymes and bile salts that make up oesophageal refluxate, which could contribute to development of pathology and VPH symptomology. Another potential drawback of using this model in healthy volunteers is that it is uncertain whether patients suffering from GORD will respond in the same way. Since it is unknown how similar these two groups are, as the cohort in question had a below average expectation trait anxiety when compared to similar studies (297-299), it would thus be unwise to generalise results obtained, and further study in this regard is warranted. Finally, even though comparable results were found between the different research centres, successful deep breathing is experimenter dependent as a more experienced experimenter may cause a bigger effect size.

3.6 Conclusions

In conclusion, study 4's findings represent the first human studies addressing the pivotal role of the PNS in mediating visceral pain hypersensitivity. It has been shown that the induction of acid-induced hypersensitivity in the proximal oesophagus in a human model of visceral hypersensitivity is altered by physiological and pharmacologically influencing PNS tone. These findings strongly indicate that the PNS plays a central role in the development of central sensitisation. Further study is now required to investigate the potential of therapeutically manipulating PNS tone in the management of chronic visceral pain syndromes.

Study 4 is the first human study to assess the role of parasympathetic nervous antagonism using atropine in modulating acid induced oesophageal pain hypersensitivity. Future directions could look into whether diminished vagal activity does exist in patients with GORD, which might explain whether it does contribute to clinical oesophageal

acid sensitivity. Since the results have demonstrated that there is an autonomic response noted with acid infusion, it would be of value to test this in patients with GORD to determine whether there is greater sympathetic dominance in this patient group.

Despite laudable progress in gastrointestinal neuroscience research directed towards describing the culpable mechanisms that account for development of visceral pain, in conjunction with considerable investment in drug development, translation into tangible improvements in patient outcomes have remained poor. (51, 338) Moreover, given that the contemporary pharmacological armamentarium has limited efficacy, and in some cases marked concerns regarding safety (339), it comes as no surprise that the multidisciplinary approach utilising a number of psychosocial and psychophysiological treatments have been used in the treatment of visceral pain. (340, 341) The results of study 4 could also be applied clinically by using the deep breathing intervention in patients undergoing biofeedback training for pain-related diseases. Deep breathing techniques may be used in a variety of chronic pain states, which are characterised by clear limitations in drug treatment, and can be tailored to the individual needs of each patient. Furthermore, since the modulation is physiological rather than pharmacological, the treatment is not associated with any negative health side effects. However, further research must also address the limitation that a decrease in experimental pain perception due to deep breathing does not necessarily mean a significant alleviation of a patient's clinical pain. This knowledge gap should now be addressed, possibly by including the study of subject groups' genetic predisposition and its contributing role in offering protection against the development of clinical hypersensitisation conditions.

6 Evidence of a role for GTP cyclohydrolase-1 in Acid Induced Oesophageal Hypersensitivity - Study 5 (Psychogenetic Pilot Study)

6.1 Introduction

The enzymatic conversion of guanosine triphosphate (GTP) to neopterin by GTP cyclohydrolase-1 (GCH-1) is a rate-limiting step in the de novo synthesis of tetrahydrobiopterin (BH4) (Figure 105), a co-factor for the production of monoamines and nitric oxide. (342) BH4 production is normally tightly controlled, however following tissue or neuronal injury expression of GCH-1 is enhanced leading to increased production of BH4, which in turn facilitates the activation of sensory nerves. (343, 344) For example intraplantar injection of BH4 causes mechanical hyperalgesia in rodents and triggers calcium transients in isolated DRGs. (344) Furthermore substantial reduction in pain behaviours can be seen following treatment with the selective GCH-1 inhibitor, 2,4-diamino-6-hydroxypyrimidine (DAHP) or knock down of GCH-1 with small hair pin RNA in animals. (344, 345)

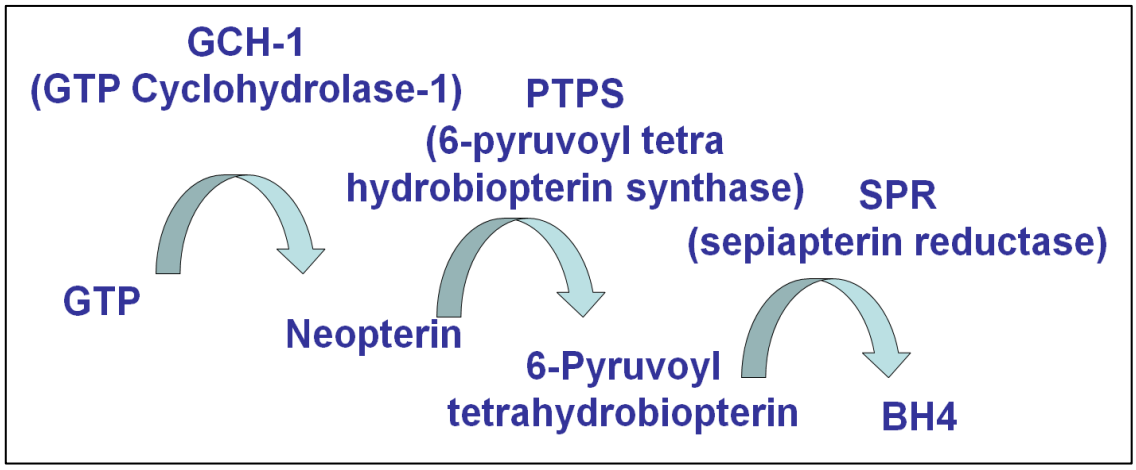


Figure 105 *Biosynthetic pathway for the de novo synthesis of BH4.*
(Adapted from Costigan, 2012) **(342)**

Importantly these preclinical observations are supported by genomic studies which have identified the presence of a GCH-1 haplotype in patients and volunteers that is associated with reduced BH4 production and reduced pain. This haplotype was originally found to be associated with lower post-operative pain scores following discectomy (344) and lower pain scores and higher pain thresholds following sensitisation in healthy volunteers. (344, 346, 347) Further studies in patients have continued to support a role for GCH-1 in pain processing, demonstrating an association between the pain protective GCH-1 haplotype and reduced analgesic requirement or delayed opioid use in chronic pain states or cancer patients, (348, 349) in addition to improved pain scores and outcomes following surgical treatment of degenerative disc disease. (350) (Figure 106) Little research has been conducted on the role of GCH-1 in visceral pain, one study has demonstrated a modest increase in the prevalence of the haplotype within a subgroup of patients with pancreatitis, (351) however the importance of this finding is unclear.

The goal of this study was to further investigate the role of GCH-1 in visceral pain. The role of the GCH-1 gene in mediating visceral analgesia was indicated in a previous preliminary GCH-1 inhibitor study of a rodent model of visceral pain. This study was performed via collaboration between Dr David Bulmer, currently a lecturer at the Wingate Institute, Queen Mary University of London, and Professor Beverley Greenwood-Van Meerveld from Oklahoma University as part of a Glaxo-Smith Kline sponsored study.

Briefly this study examined the role for GCH-1 in visceral pain by eliciting the effects of the selective GCH-1 inhibitor DAHP, on spontaneous pain behaviours elicited by colorectal injection of a chemical irritant (3% mustard oil), in male *Sprague Dawley* rats. In the rodent visceral pain

model, experimental pre-treatment with DAHP produced a substantial, and dose related inhibition of pain behaviours at doses from 10 to 180mg/kg i.p. $p<0.05$. The data generated in these studies suggested that GCH-1 played an important role in visceral pain processing and required further investigation in a healthy volunteer model of visceral pain.

Based on the results of the above study it is possible to speculate that the difference in the genotype could also further explain the inter-individual differences in pain response, observed in the human model of visceral pain. Our aim was to evaluate the possible contribution of the GCH-1 pain protective haplotype to visceral pain processing in healthy volunteers characterised for baseline oesophageal pain thresholds, sensitisation to oesophageal acidification, and psychological states of depression and anxiety. Our initial *hypothesis* being that the prevalence of the pain protective GCH-1 haplotype would be greatest in subjects who did not sensitise to acid injury by comparison with subjects that were sensitive to acid injury. As was seen in study 4, further research is needed to address the limitation found, where the decrease observed in experimental pain perception due to the "deep breathing increased CVT" component does not fully equate to a complete alleviation of a patient's clinical pain or the degree of sensitisation. Study 5 is a start in attempting to address this knowledge gap by including the examination of subject groups' psycho-genetic predisposition and its contributing role in offering protection against the development of clinical hypersensitisation conditions and its associated psychiatric sequelae. (Figure 106)

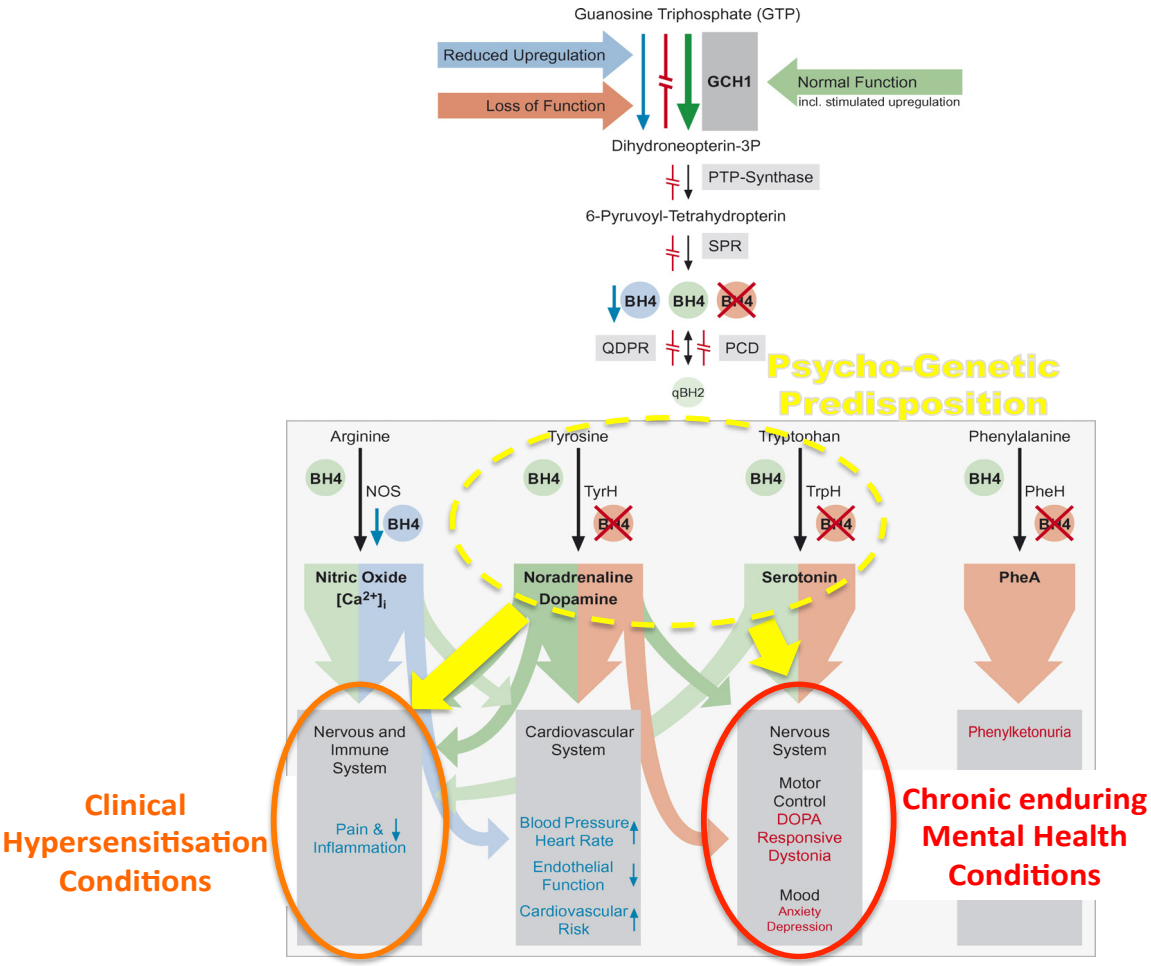


Figure 106 *Illustration of the central psycho-genetic predisposing role of BH4 and its contribution in offering protection against the development of clinical hypersensitisation conditions & its resulting mental health sequelae "Tetrahydrobiopterin pathway, with the rate-limiting enzyme GTP cyclohydrolase 1, and its functional clinical implications. GTP cyclohydrolase expression and/or activity are up regulated during inflammation, mast cell stimulation, following ischemic stroke or peripheral nerve injury leading to increased BH4 production. Excess BH4 in peripheral sensory neurons following axonal injury contributes to the manifestation of neuropathic pain. This is mediated in part by increasing calcium influx and nitric oxide production. Inhibition of GCH1 activity or reduced GCH1 upregulation reduces pain in various models. In blood vessels BH4 is required to produce nitric oxide by endothelial NOS (eNOS). Relative BH4 deficiency leads to an uncoupling of oxidation–reduction steps performed by eNOS resulting in increased production of reactive oxygen species, instead of nitric oxide, that contribute to endothelial dysfunction. Increasing endothelial BH4 improves vascular functions, particularly in diabetes models. In the brain BH4 is required for the production of dopamine and serotonin. BH4 deficiency due to loss-of-function mutations of GCH1 lead to DOPA-responsive dystonia, a Parkinson-like neurologic disease, or to atypical phenylketonuria. On the other hand, excess BH4 in the striatum contributes to the dying of dopaminergic neurons probably mediated by enhanced calcium influx and disturbance of the redox balance. Similarly, excess BH4 after stroke due to GCH1 upregulation contributes to neuronal death. [Abbreviations: GTP, guanosine triphosphate; GCH1, GTP cyclohydrolase 1; PTPS, 6-pyruvoyl tetrahydropterin synthase; SPR, sepiapterin synthase; QDPR, quinoid dihydropteridine reductase; PCD, pterin-4a-carbinolamine dehydratase; BH4, tetrahydrobiopterin; BH2, dihydrobiopterin; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PheH, phenylalanine hydroxylase; PheA, phenylalanine; TyrH, tyrosine hydroxylase; DA, dopamine; NA, noradrenaline; 5-HT, serotonin; TrpH, tryptophan hydroxylase; ONOO, peroxynitrite; BP, blood pressure; CAD, cardiovascular disease."] Quoted and adapted from Doering (2008) (352)*

6.2 Materials and Methods

6.2.1 Oesophageal pain testing

Based on a clear reduction in visceral pain behaviours seen in the animal model following pre-treatment with DAHP, we went on to evaluate the potential contribution of the pain protective GCH-1 haplotype to visceral pain thresholds and depression and anxiety scores in healthy volunteers who were subjected to psychological profiling and oesophageal pain testing using a previously well validated model of acid induced central sensitisation. (184-190) The study was approved by the 'East London and The City Research Ethics Committee - Alpha' (ref: 09/H0704/71) and all subjects provided written informed consent prior to the start of the experiments.

6.2.2 Protocol

Volunteers underwent oesophageal pain testing during two visits separated by 2-4 weeks as part of a cross over design interventional study. (Sham breathing visit protocol – Study 2 & 3, see section 2.20.2, page 111) Volunteers were randomly assigned to an intervention prior to oesophageal acidification on either their first or second visit. Only data generated prior to intervention or on the non-interventional visit was used for this study. In addition during the first visit volunteers underwent psychological profiling with hospital-based depression and anxiety based questionnaires following which blood samples were taken for genomic analysis and frozen down.

6.2.3 Other Methods of Measurement

All other methods of measurement; Catheter Assembly[18] (section 2.4, page 78), Oesophageal acid infusion (section 2.4, page 78), Oesophageal pH monitoring (section 2.6, page 80), Pain Threshold Measurements (section 2.8, page 82), Psychological assessment (section 2.11, page 85) and Measurement of the Autonomic Nervous System[19] (section 2.12, page 86) was performed as described in their specific sections.

6.2.4 Genotyping and pain phenotyping

GCH-1 haplotype was examined in blood samples in 38 healthy volunteers from the original study cohort of 72 which could be classified as sensitisers (n=19, mean age 27yrs; 11 females) or non-sensitisers (n=19, mean age 27yrs; 11 females) based on changes in their proximal oesophageal pain threshold to electrical stimulation following acidification of the distal oesophagus during their non-interventional visit. For the purposes of this study non sensitisers were characterised by a decrease in pain threshold no greater than 5mA or an increase in pain threshold following acidification (mean post acid increase of 2.4 ±1.4mA), and sensitisers were characterized by a decrease in pain threshold of 15mA or greater (change mean post acid decrease of 22.6 ±2.0mA). (Figure 107) The remaining subjects had a change in pain threshold >5 and<15mA following acidification and were not genotyped. [20]

[18] For a more detailed description see appendix one.
[19] For a more detailed description see appendix one.
[20] For a more detailed description appendix four.

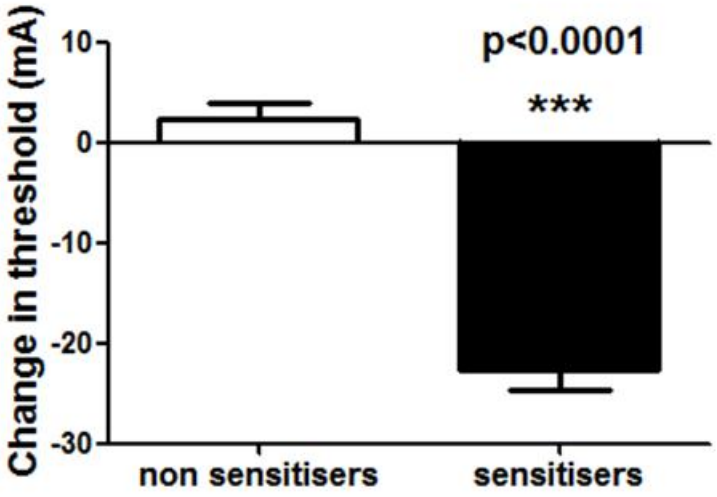


Figure 107 ***Human acid infusion study showing ∆ pain threshold following acidification with (white) non-sensitisers (mean post acid increase of 2.4 ±1.4mA), and (black) sensitisers (mean change mean post acid decrease of 22.6 ±2.0mA).***

Blood samples from these subjects were thawed, genomic DNA extracted and genotyped for the previously described pain protective GCH-1 haplotype using a 3 SNP screening strategy devised by Lotsch *et al.* (2007), which identifies the haplotype with 100% accuracy. Taqman assay kits were used to genotype for the three SNPs (dbSNP rs8007267G>A in the 50 untranslated region, rs3783641A>T in intron 1, and rs10483639C>G in the 30 untranslated region spanning the entire GCH1 gene range) in a 384 well format using 5ng genomic DNA from each patient, total reaction volume 5μl. CGH haplotypes were reconstructed using Haploview. (353) [21]

[21] For a more detailed description appendix four

6.2.5 Data analysis

Subjects were stratified by GCH-1 haplotype into those possessing at least one allele for the pain protective haplotype (X) or not (O). In addition subject's pain phenotype was also determined from the change in pain threshold post acid infusion by calculating the mean value of the pain thresholds 30, 60 and 90 min post acid infusion and subtracting the pre-acid pain threshold. Subjects were stratified into sensitisers and non-sensitisers based on this value as described above. Baseline oesophageal pain thresholds on first and second visits, the change in pain threshold between first and second visits, depression and anxiety scores were then compared between subjects grouped by haplotype, pain phenotype and a combination of haplotype and pain phenotype using Student's t-test or one way ANOVA as appropriate, significance set at $p<0.05$. Additionally the prevalence of GCH-1 haplotype was compared between sensitisers and non-sensitisers using Fisher's exact test. All data is expressed as mean ±SEM unless otherwise stated.

6.3 Results

6.3.1 Prevalence of the pain protective GCH-1 haplotype (X):

The allelic frequency of the pain protective haplotype was 0.18 (14/62) across all 38 subjects genotyped, which was comparable with reported values in the literature. This resulted in the presence of n=12 volunteers heterozygous (O/X) for the haplotype and n=1 individual homozygous (X/X) for the haplotype. No difference was seen in the prevalence of the pain protective haplotype between subjects classified as sensitisers compared with non-sensitisers. (Figure 108)

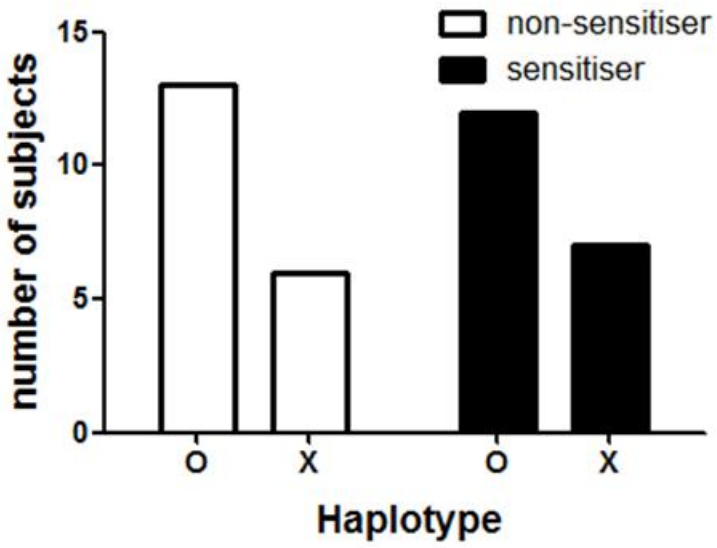


Figure 108 *Pain thresholds and haplotype/phenotype. (n=12) volunteers heterozygous (O/X & homozygous (X/X) for the haplotype. No difference was seen in the prevalence of the pain protective haplotype sensitisers & non-sensitisers.*

6.3.1 Characterisation of oesophageal pain thresholds by GCH-1 haplotype:

Across all 38 subjects baseline oesophageal pain thresholds were greater on the second visit compared with the first (e.g. 44.6 ± 3.3mA vs. 57.1 ± 3.9mA first vs. second visit $p<0.001$). However no significant difference was seen in pain thresholds based on the presence or absence of the pain protective haplotype during either visit (e.g. first visit 44.9 ±4.2(SEM) vs. 44.0 ±5.6(SEM) $p=0.90$; second visit 55.8 ±4.7(SEM) vs. 59.5 ±7.2(SEM) $p=0.66$; (O) n=25 vs. (X) n=13 respectively), and no difference in the change in threshold between visits was seen based on haplotype (e.g. 11.8 ±2.0mA vs. 16.4 ±5.2mA $p=0.33$ (O) vs. (X). Similarly no significant difference was seen in baseline oesophageal pain thresholds if subjects were stratified based on both haplotype and pain phenotype, although a trend towards a greater pain threshold was seen on the second visit in

sensitisers who possessed the pain protective haplotype compared with sensitisers who did not (e.g. 77.6 ±6.2mA vs. 60.6 ±6.1mA p=0.087; (X) n=7 vs. (O) n=12). (Figure 109)

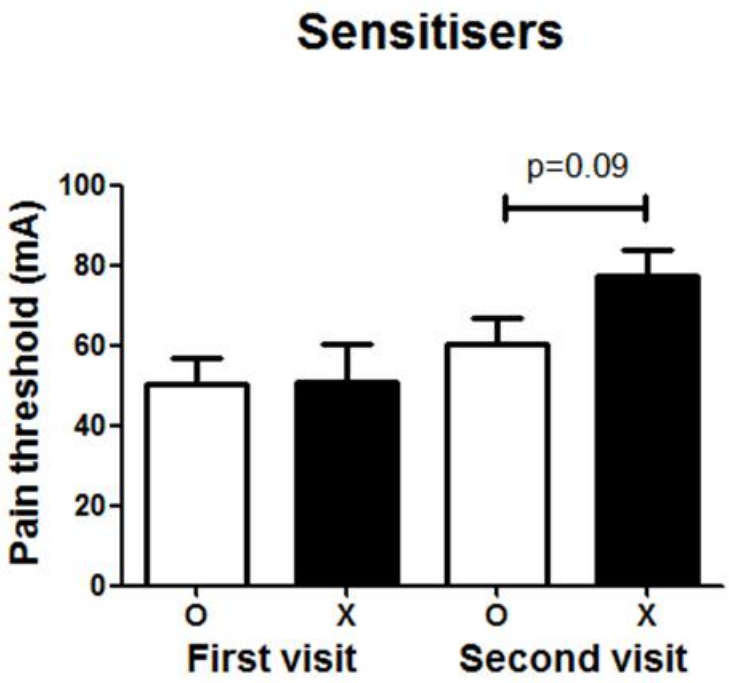


Figure 109 ***Baseline oesophageal pain thresholds. Comparing first & second visits. PT's were greater on the second visit compared with the first (77.6 ±6.2mA vs. 60.6 ±6.1mA p=0.087; (X) n=7 vs. (O) n=12)***

Analysis of the change in pain threshold between visits did however reveal a significantly greater increase in threshold for sensitisers that possessed the haplotype compared with sensitisers who did not and both subgroups of non-sensitisers (e.g. 26.6 ±6.2mA (X) sensitiser n=7 p=0.012 vs. 10.1 ± 2.4mA (O) sensitiser n=12 vs. 4.5 ± 5.9mA (X) non-sensitiser n=6 vs. 13.6 ± 3.1mA (O) non-sensitiser n=12). (Figure 110)

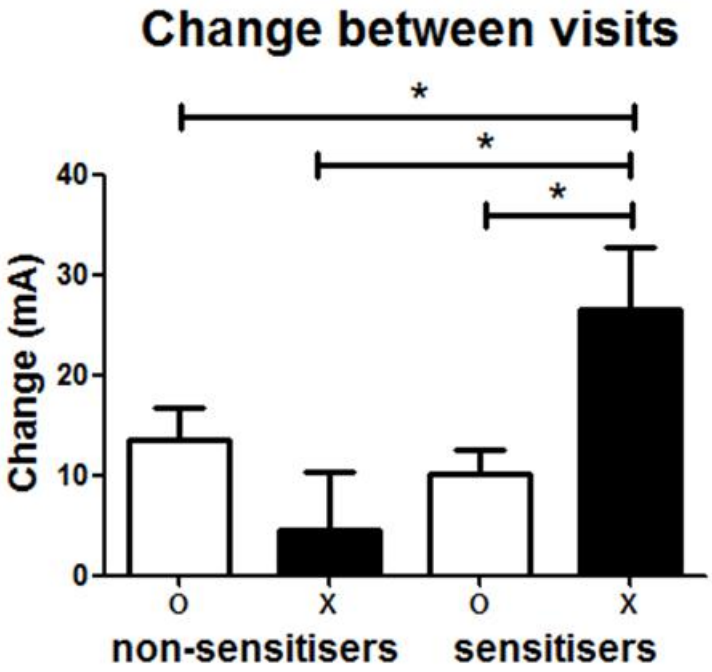


Figure 110 ***Analysis of the change in pain threshold between visits and pain protective haplotype.***

6.3.2 Characterisation of depression and anxiety scores by GCH-1 haplotype:

No difference was observed in depression or anxiety scores based on haplotype (depression 9.0 ±0.3(SEM) vs. 8.3 ±0.5(SEM) p=0.23; anxiety 8.6 ±0.5(SEM) vs. 9.4 ±0.7(SEM) p=0.37; (O) vs. (X) respectively). However in a comparable manner to pain thresholds, analysis of depression scores based on pain phenotype and genotype revealed that depression scores were significantly lower in sensitisers, Δ2.11 ±0.62(SEM), p=0.008 (Figure 111) who possessed the pain protective haplotype compared to sensitisers who did not or both subgroups of non-sensitisers (e.g. depression score of 7.1 ±0.5(SEM) (X) sensitisers p=0.03 vs. 9.3 ±0.4(SEM) (O) sensitisers vs. 9.7 ± 0.7(SEM) (X) non sensitisers vs. 8.8 ±0.5mA (O) non-sensitisers). (Figure 112) No difference was observed in anxiety scores

when subjects were grouped by pain phenotype and haplotype. (Figure 113)

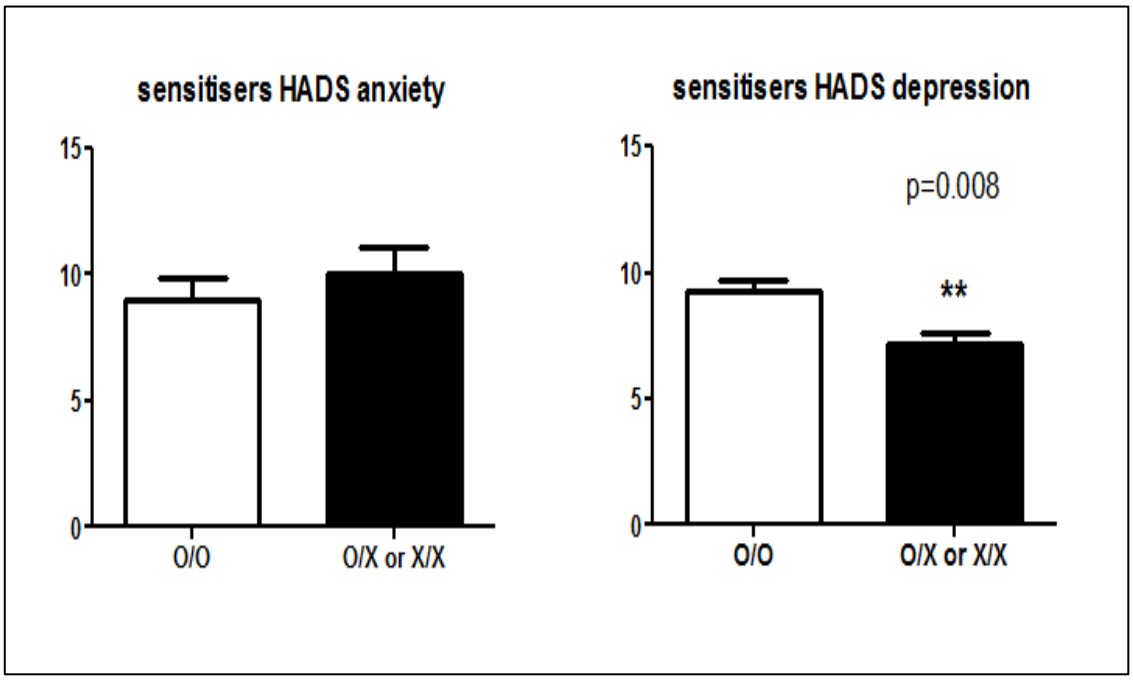


Figure 111 ***Analysis of the sensitisers' HADS*** **– Anxiety &** ***Depression scores and haplotype /phenotype.***

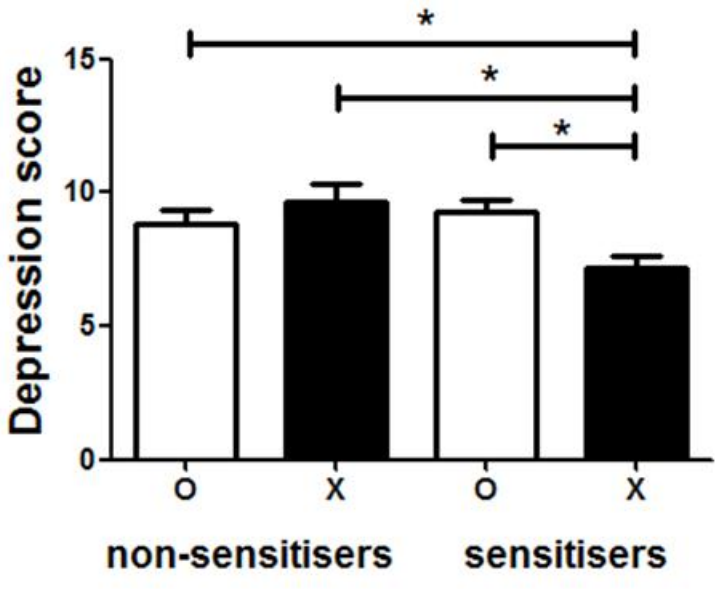


Figure 112 *Analysis of HADS - Depression scores and haplotype/phenotype.*

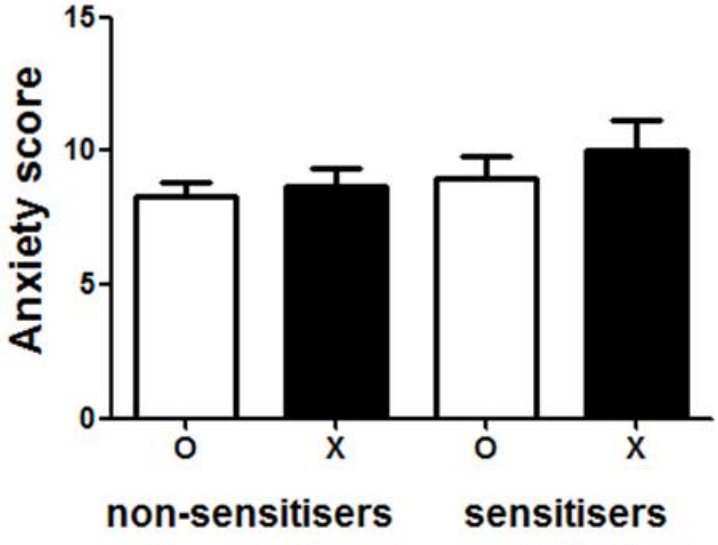


Figure 113 *Analysis of HADS - Anxiety scores and haplotype/phenotype.*

6.4 Discussion

With this study we have generated data that supports a role for GCH-1 in visceral pain processing. We found a significant increase in pain thresholds on repeat pain testing in a subgroup of subjects who demonstrated robust central sensitisation to oesophageal injury and possessed the pain protective GCH-1 haplotype.

Utilising a visceral model of central sensitisation by acidifying the distal oesophagus and examining pain thresholds within the proximal oesophagus. This restricts our observations to the resultant secondary hyperalgesia elicited in response to the central sensitisation produced by injury of the distal oesophagus. (354) Additionally by measuring pain thresholds with electrical stimulation we further restrict our observations to central changes by bypassing the contribution of stimulus transduction mechanisms to the activation of nociceptors within the proximal oesophagus. Two pain phenotypes are typically seen following oesophageal acidification, subjects who sensitise and present lower pain thresholds following injury (sensitisers), and subjects who do not sensitise and retain comparable pain thresholds following injury (non-sensitisers). Data from human studies has shown that pain thresholds following sensitisation of the skin in healthy volunteers were increased in subjects who possessed the GCH-1 pain protective haplotype consistent with a role for GCH-1 in inflammatory pain. (344, 346, 347) We therefore hypothesised that subjects with this GCH-1 haplotype may also have a reduced response to acid injury of the oesophagus. The prevalence of the pain protective haplotype would therefore be more in non-sensitisers compared with sensitisers.

The data generated in the current study did not however support this hypothesis. The prevalence of the pain protective haplotype was

comparable between sensitisers and non-sensitisers. Furthermore baseline pain thresholds were also comparable between subjects with the pain protective haplotype and those without. However if pain thresholds were examined by pain phenotype and genotype we did find a trend towards increased pain thresholds on the second visit between sensitisers who possessed the pain protective haplotype and sensitisers who did not possess the haplotype. Furthermore a substantial increase in baseline pain thresholds was seen between first and second visits in these sensitisers who possessed the pain protective haplotype which was significantly greater than sensitisers who did not possess the haplotype and non-sensitisers regardless of whether they possessed the haplotype or not. Interestingly a similar pattern was found when depression scores were examined with the exception that depression scores were significantly lower in subjects possessing the pain protective haplotype and who sensitised to oesophageal acidification compared with the other groups.

Further studies are now needed to confirm the validity of our initial findings. The difference in pain thresholds and depression scores within subjects grouped by GCH-1 haplotype and pain phenotype suggests the two findings may be related. For example subjects with lower depression scores may adapt more quickly to the prospect of a repeat pain test and hence show increased pain thresholds. However it is not clear why we have only found these differences in a subgroup of subjects who possessed the pain protective GCH-1 haplotype and develop secondary hyperalgesia to injury as opposed to all subjects with the haplotype.

One explanation for our changes is that subjects who sensitise following acid injury to the oesophagus have an on going contribution by GCH-1 to their baseline pain thresholds which is reduced in people with the pain

protective haplotype and hence results in their higher pain thresholds. By contrast in subjects who don't sensitise to injury there is little or no GCH-1 contribution to baseline pain thresholds and so baseline pain thresholds are unaffected by the presence or absence of the pain protective GCH-1 haplotype. Precisely what this might be is unclear, however the lower depression scores observed in the same subgroup of subjects who sensitise and possess the pain protective haplotype suggests that a link to emotional states may be important. This can be expected because of the polygenic nature of hypersensitisation conditions and its resulting mental health effects, which are additionally shaped by psychological and environmental pressures. (355, 356) With modest phenotypic consequences such as for pain, variants in other genes are likely to contribute to the phenotype to a similar extent, sometimes with opposite or cancelling out effects as proposed by Lötsch *et al*. (357). This might also be a reason for non-reproductions of genetic associations in polygenically controlled symptoms, as observed in this study for the pain-protection by GCH1 variants. (358, 359)

6.5 Conclusion

The goal of this study was to further investigate the role of GCH-1 in visceral pain. The role of the GCH-1 gene in mediating visceral analgesia was indicated in a previous GCH-1 inhibitor study of a rodent model of visceral pain. The difference in the genotype could also further explain the inter-individual differences in pain response, observed in the human model of visceral pain. Our aim was to evaluate the possible contribution of the GCH-1 pain protective haplotype to visceral pain processing in healthy volunteers, with the hope of replicating its pain protection. Although we did not find a clear association between the GCH-1 pain protective haplotype and sensitisation of the acidified oesophagus, a

modest psychological effect was observed with regard to depression scores, highlighting the difficulties of research of polygenic conditions affecting hypersensitisation. Study 5 represents a start in attempting to address the knowledge gap with regard to the psycho-genetic predisposition and its contributing role in offering protection. Further research is now needed to address the existing limitations in our understanding of the development of clinical hypersensitisation conditions and its associated psychiatric sequelae.

7 Summary and General Discussion

The aim of my research programme in essence was to ascertain what the determinants and ANS mechanisms were for predicting the inter-individual differences with regard to the vulnerability of developing acid-induced oesophageal pain hypersensitivity (OPH), and to determine if modulation of these ANS mechanisms could influence the degree of acid-induced OPH.

7.1 Introduction and study rational

Studying the factors that influence the development of post-injury visceral pain hypersensitivity is important, as I was aware that clinically the majority of individuals recover after an episode of visceral inflammation or injury, but a proportion go on to develop a functional gastrointestinal disorder with demonstrable visceral pain hypersensitivity (VPH). (41) This suggests phenotypic differences in the way individuals respond to and recover from injurious stimuli in the viscera. Reviewing preliminary research, I became aware of the inter-individual variability in magnitude of sensitisation to acid in the model. The factors underlying why some individuals developed greater sensitisation compared to others, and why some failed to sensitise at all, were not known. It was clear that understanding these factors might help understand the mechanism of injury-induced visceral pain hypersensitivity. Furthermore, identifying biological differences between individuals that predicted their tendency to sensitise to acid in the model might help identify phenotypic traits that predispose to or protect against injury-induced visceral sensitisation. This in turn might identify new targets for therapeutic developments. To achieve my aim I performed a number of studies in healthy human volunteers (chapters 3-6) using a previously well-

validated model of acid-induced OPH. (177, 184-190) Autonomic nervous system activity during infusion was measured continuously in real time with novel technology that derived markers of selective sympathetic and parasympathetic activity.

7.2 Summary of what I have demonstrated

7.2.1 Chapter 3 (study 1 – pilot study)

As study 1 was a pilot study and not fully powered the emphasis was on identifying early trends that would be investigated more thoroughly in the subsequent studies, and to test the suitability of the various psychophysiological modulations proposed in this model. It was hypothesised that sensitisation as expressed by the difference in average pain threshold (ΔPT) would be directly proportional to sympathetic nervous system activation (SNS: ΔSCR), and parasympathetic nervous system withdrawal (PNS: ΔCVT), as induced or amplified by different psychophysiological modulations. The secondary aim of the study was to expand on the data in order to determine what psychological state and trait factors predicted the degree of sensitisation to acid in the model.

I found that in spite of all subjects being acid infusion naïve, 22% did not sensitise during acid infusion, and also failed to sensitise on subsequent visits, irrespective of modulation. These non-sensitisers demonstrated variable acid-induced autonomic responses between the different experiments. For these subjects the degree of sensitisation between visits was not related to the degree of change in HR, CVT or CSB. They had less sympathetic activation (SCR) compared to sensitisers, and scored less for neuroticism on the BFI. Overall their response pattern suggested that they might represent a distinct phenotype with reduced

susceptibility to injury-induced sensitisation in this model. As a previous study using this model demonstrated that stress induction increases the degree of secondary oesophageal hyperalgesia in sensitisers, (30) it now remained to further investigate this phenomena, to ascertain if this could be replicated in the non-sensitiser group.

The following conclusions were reached as a result of study 1:

1. The Isometric "handgrip" exercise test was not suitable for trying to increase sympathetic tone, as it produced both an initial parasympathetic withdrawal and then rebound increase.
2. Due to subjects' anxiety habituation with potential for decreasing induction of acid induced sensitisation on subsequent visits, the number of visits should be kept to a minimum. The screening visit was hence discontinued, and randomisation occurred directly following recruitment.
3. The Deep breathing modulation was successful in producing visceral desensitisation in this model. But due to non-specific factors associated with deep breathing it was decided to test it against an active placebo.
4. The inter-individual variability in the magnitude of sensitisation between sensitisers and non-sensitisers should now be further explored in a comparative study that will allow the evaluation of ANS responses across a spectrum of experimental stressors.

7.2.2 Chapter 4 (study 2 - deep breathing in sensitisers & study 3 – stress induction in non-sensitisers)

The results from studies 2 and 3 represented the first human studies addressing the pivotal role of the ANS in mediating VPH using this model

of OPH. Study 2 (fully powered hypothesis testing study) provided evidence for how sensitisation can be prevented by deep breathing through its action on increasing NA mediated - PNS tone, and study 3 (hypothesis generating pilot study) demonstrated the mechanistic paradoxes with regards to ANS regulation across a continuum of experimental stress levels.

7.2.2.1 Chapter 4 *(study 2 - deep breathing in sensitisers)*

Study 2's results represent a novel human intervention study addressing the key role of the Nucleus Ambiguus (NA) mediated - PNS in regulating visceral pain hypersensitivity. It demonstrated how acid-induced hypersensitivity could be abolished by physiologically increasing PNS tone. This finding strongly indicates that the PNS plays a central role in the development of central sensitisation. It was now important to study the effect of co-administered atropine and deep breathing on sensitisation. This could potentially examine and contrast the contribution and importance of the neurobiological pathways that underlie deep breathing induced PNS analgesia. As the placebo response is a manifold phenomenon, the analgesia that was observed in study 2 could plausibly be due to the observed reduction in anxiety. Also study 2's results needed to be independently validated by means of an unrelated cohort in another study centre.

7.2.2.2 Chapter 4 *(study 3 – stress induction in non-sensitisers)*

The factors associated with failure to sensitise in the model were poorly understood; it has been noted that around 1 in 5 subjects display this. (175) Previous studies that were performed to pharmacologically modulate hyperalgesia using this model and help understand the receptor mechanisms of central sensitisation excluded non-sensitisers to

participation. (179, 180, 185) As such it was not known why they failed to sensitise and whether they sensitised on subsequent studies. Therefore, their selective study during repeated acid exposures was necessary to examine the consistency of response.

Study 3's results provided novel evidence that explain and clarify for the first time the hitherto poorly understood multifactorial ANS regulatory mechanisms of visceral pain hypersensitivity in subjects who fail to sensitise to acid infusion. With the coinciding 'real time' examination of all three parts of the biopsychosocial model in this study, it allowed for an important novel synthesis to be made between developmental psychology, neurobiology and gastroenterology. This allowed us to reinterpret previously conflicting results with more clarity and potentially greater therapeutic advantages. With the incorporation of attachment and polyvagal theory, study 3's results demonstrated the paradoxes surrounding ANS regulation with regard to central sensitisation as influenced by differing environmental stress assessments. It also highlighted the need for a deeper understanding of the vulnerability phenotypes involved.

7.2.3 Chapter 5 (study 4 - placebo controlled /atropine challenge study)

Study 4 (hypothesis testing study) was the first human study to assess the role of parasympathetic nervous system antagonism using atropine in modulating acid induced OPH, and it independently validated study 2's results by means of an unrelated cohort in another study centre.

In study 4, it was observed that in both arms of the study there was modulation of the induced hyperalgesia. Thus In the atropine arm, where

CVT's effect was antagonised, a degree of desensitisation also occurred in comparison to the sensitisation that was observed in the screening visit. In other words, factors other than the increase in CVT were involved in reducing visceral sensitisation. This could be due to the 'non specific' therapeutic elements of the behavioural intervention, but also that of 'placebo effect' induced as a result of the subjects being aware of an intervention. The placebo arm of the study resulted in subjects receiving the un-atropinised increase in CVT (i.e. the active treatment).

However because in study 4 the 'placebo arm's paced deep breathing' had efficacy over and above the effects of the atropine's 'antagonised' deep breathing arm's response, in spite of also being exposed to the nonspecific therapeutic elements of distraction and increased interpersonal interaction while being "paced", this strongly suggested that the activation and increase of the NA mediated CVT by the deep breathing's RSA added additional reduction in acid induced sensitisation and hyperalgesia of the spinal dorsal horn mediated central sensitisation *per se*, and thus could potentially provide additional 'direct' clinical efficacy in symptom reduction due to VPH. These findings thus confirmed that the PNS plays a central role in the development of central sensitisation.

7.2.4 Chapter 6 (study 5 – psycho-genetic pilot study)

The goal of this study (hypotheses generating pilot study) was to further investigate the role of GCH-1 in visceral pain. The role of the GCH-1 gene in mediating visceral analgesia was indicated in a previous GCH-1 inhibitor study of a rodent model of visceral pain. The difference in the genotype could also further explain the inter-individual differences in pain response, observed in the human model of visceral pain. My aim

was to evaluate the possible contribution of the GCH-1 pain protective haplotype to visceral pain processing in healthy volunteers, with the hope of replicating its pain protection. Although we did not find a clear association between the GCH-1 pain protective haplotype and sensitisation of the acidified oesophagus, a modest psychological effect was observed with regard to depression scores, highlighting the difficulties of research of polygenic conditions affecting hypersensitisation. Study 5 represents a start in attempting to address the knowledge gap with regard to the psychogenetic predisposition and its contributing role in offering protection. Psychogenetic-neurogastroenterology is presently in its infancy, and further research of this kind is clearly indicated.

7.3 Psychophysiological Mechanisms in acid induced OPH

The studies presented in this thesis have demonstrated that acid-induced oesophageal pain hypersensitivity can be modulated by a number of psychophysiological factors. The mechanisms underlying how sensitisation develops after acid exposure in the model are now better understood and indicate that it is a combination of peripheral and central factors.

7.3.1 Psychophysiological Mechanisms in Sensitisers and non sensitisers

In sensitisers acid infusion may activate acid-sensing receptors such as TRPV1 and ASIC resulting in increased primary afferent activity. The increased nociceptor barrage onto spinal dorsal horn neurones may activate a number of receptors, including NMDA receptors, resulting in the development of central sensitisation, further enhancing nociception. The magnitude and duration of central sensitisation that develop are

modulated by supraspinal pain inhibitory and facilitatory systems that in turn are influenced by cognitive factors such as psychological state, adult attachment vulnerability, alexithymia scores, attention & arousal levels and anticipation.

In non-sensitisers the mechanisms in the model remain speculative. A failure of sensitisation once again could be due to peripheral or central effects. Non-sensitisers may have enhanced mucosal barrier function in response to injurious stimuli, which might result in reduced nociceptive inputs to the spinal cord. As a result, the magnitude of central sensitisation that develops in response to peripheral insults may be attenuated. Alternatively, these individuals may have enhanced supraspinal inhibitory processes that either reduce the magnitude of injury-induced central sensitisation at dorsal horn level, or inhibit the transfer of nociceptive inputs to cortical centres where pain is evaluated, and will be further considered below in contexts of supraspinal ANS stress response mediation. It might be possible to test the involvement of endogenous opioid systems in these individuals by determining whether naloxone induces hyperalgesia after acid in these individuals.

My work has however provided novel insights into how the ANS may differentially modulate the response to oesophageal acidification in sensitisers and non-sensitisers. In the following section I provide an interpretation of my results in sensitisers versus non-sensitisers within the conceptual framework provided by Porges poly vagal theory described in detail in sections 1.9.4, (page 53); section 2.12.4 (page 91) & chapter 4, table 9, (page 201).

7.4 Novel ANS responses in the context of environmental stress

Stress and anxiety have been associated with the onset and severity of symptoms in functional gastrointestinal disorders; in particular, life events associated with stress and anxiety at the time of gastroenteritis increases the likelihood of developing symptoms of IBS. (255)

7.4.1 Parasympathetic nervous system (PNS) stress response

In a safe (supportive) environment (deep breathing) both sensitisers (Figure 114, pink graph) and non-sensitisers (green graph) increase PNS activation, due to paced breathing - NA activation (240)

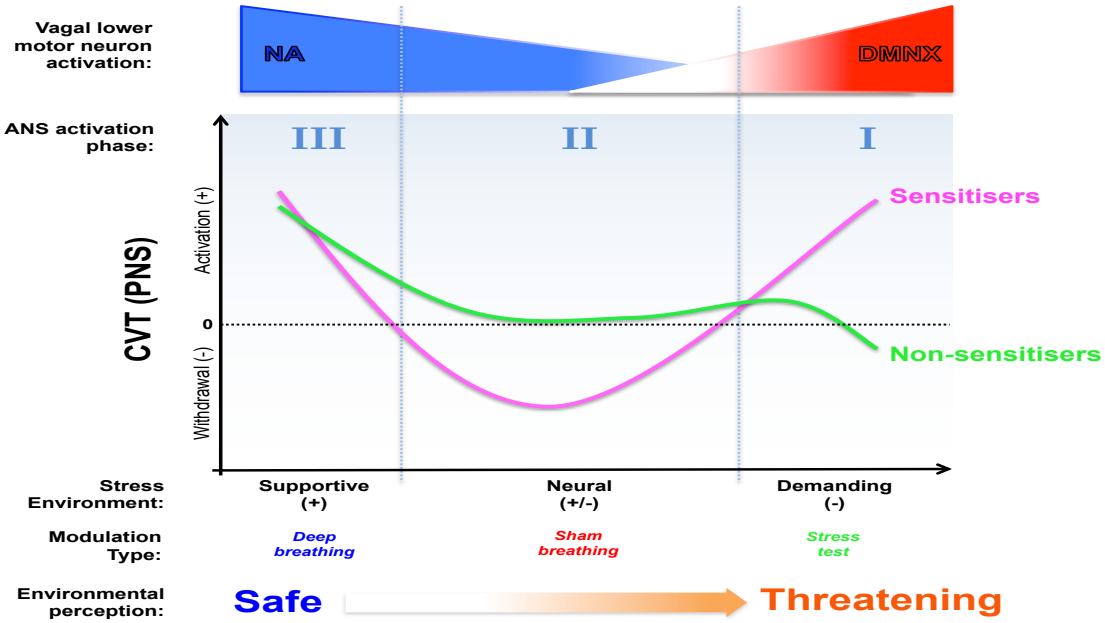


Figure 114 Illustrates the changes in Cardiac Vagal Tone (CVT) of the Parasympathetic nervous system (PNS) across three different environmental stress conditions ranging from 'supportive/safe' (left) as experienced during the deep breathing-experimental modulation procedure, threw 'neutral' (middle) as experienced during the sham breathing-experimental modulation procedure, to 'demanding/threatening' (right) as experienced during the stress test-experimental modulation procedure. This gives rise to three distinct different activation patterns as described by S. Porges (100) and illustrated by the roman numerals: III, II & I, coinciding with different vagal lower motor neuron activation, illustrated above as ranging from left, mainly Nucleus Ambiguus (NA) to the Dorsal motor neuron nucleus of the vagus (DMNX) on the right. In the foreground is a schematic representation of the changes in stress responses as observed during studies 2 & 3, for the sensitisers (pink graph), and the non-sensitisers (green graph) to acid infusion induced oesophageal pain hypersensitivity (OPH).

(*Porges-Stage III* (see table 9, chapter 4, page 201)) that desensitises visceral pain thresholds in both groups. In a neutral (sham breathing), though challenging (experimental) environment (*Porges-Stage II*) non-sensitisers are able to maintain the 'protective' vagal tone, while the sensitisers withdraw their 'protective' NA tone. In a stressful (demanding/threatening) environment (*Porges-Stage I*), the sensitisers increase their 'damaging' vagal tone (with SNS co- activation) (134-137), most likely due to DMNX activation (108), while the non-sensitisers do not.

7.4.2 Sympathetic nervous system (SNS) stress response

In a safe (supportive) environment both sensitisers (Figure 115, pink graph) and non-sensitisers (green graph) have low activation, most likely due to the regulating "vagal-brake" (143) (via - NA activation; *Porges-Stage III* (see table 9, chapter 4, page 201)), which is more efficient and neuro-chemically "cost-effective" in maintaining homeostasis. (138) In a neutral, though challenging (experimental) environment i.e. during sham breathing (*Porges-Stage II*) both the sensitisers and non-sensitisers increase SNS tone by the withdrawal of the external constraint due to the opposing "vagal-brake", but the non-sensitisers are better at mobilising SNS tone. In a stressful (demanding/threatening) environment, the non-sensitisers are more able to significantly increase their SNS tone in comparison with the sensitisers. The sensitisers are most likely unable to match this due to their DMNX co-activation (*Porges-Stage I*) that is impeding a more appropriate adaptive increased SNS response. (108)

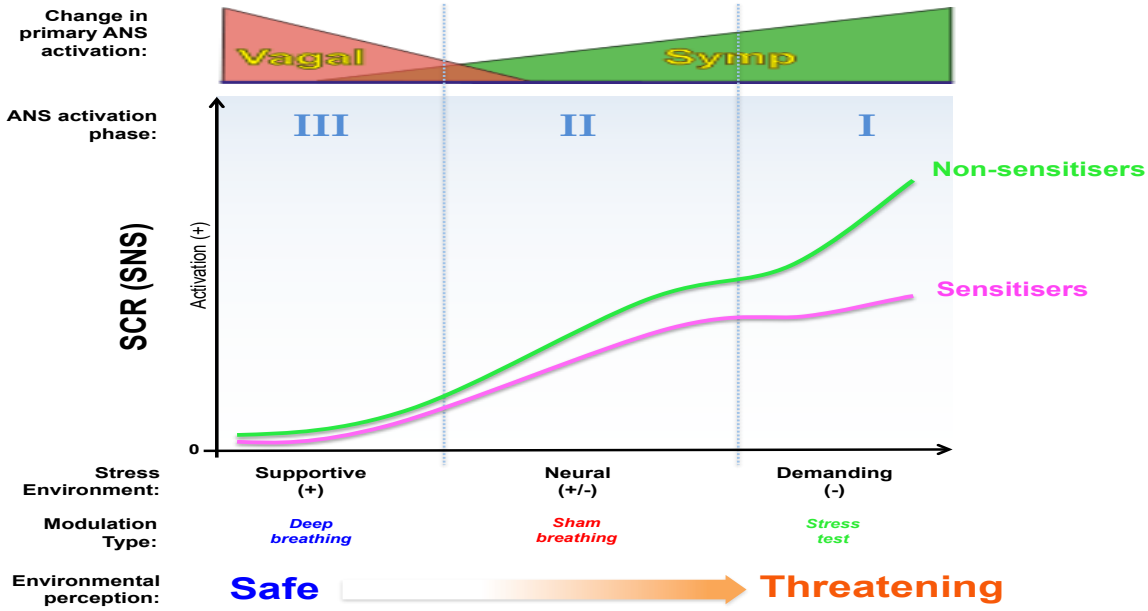


Figure 115 Illustrates the changes in sudomotor activation of the Skin Conduction Response (SCR) under control of the Sympathetic nervous system (SNS) across three different environmental stress conditions ranging from 'supportive/safe' (left) as experienced during the deep breathing-experimental modulation procedure, threw 'neutral' (middle) as experienced during the sham breathing-experimental modulation procedure, to 'demanding/threatening' (right) as experienced during the stress test-experimental modulation procedure. This gives rise to three distinct different activation patterns as described by S. Porges (100) and illustrated by the roman numerals: III, II & I, coinciding with change in primary autonomic nervous system (ANS) activation, illustrated above as ranging from left, mainly Vagal (also known as the 'vagal brake' to unimpeded sympathetic activation on the right. In the foreground is a schematic representation of the changes in stress responses as observed during studies 2 & 3, for the sensitisers (pink graph), and the non-sensitisers (green graph) to acid infusion induced oesophageal pain hypersensitivity (OPH).

7.5 The central role of stress and anxiety in the development of sensitisation in this model of OPH

In chapter 3, 4 and 5, it was demonstrated that acid infusion was associated with an increase in subjective anxiety levels, and during acid infusion there was an associated increase in sympathetic and reduction in parasympathetic activity during screening/sham breathing (Porges-stage II) in those subjects that sensitised to acid. This suggests that psychological factors can modulate the perception of pain directly at the level of the viscera. In addition it is inferred that anxiety at the time of

injury may have an additive effect on nociception that predisposes some individuals to chronic sensory dysfunction.

In chapter 5 - study 3, the novel observation where stress induction was associated with greater acid-induced sensitisation in certain individuals that previously did not sensitise to acid, replicates findings seen in sensitising subjects of previous studies using this model. (175, 179, 194) It is difficult to know how stress and anxiety has this effect as it can both modulate peripheral mucosal barrier function and permeability, (360) and have a variety of central effects. Stress induction may induce activity in certain brain regions such as the ACC that may in turn enhance pain perception. (361) Stress induction may also influence nociception through the modulation of supraspinal pain inhibitory and facilitatory systems, or exert effects through the associated reduction in vagal tone as demonstrated during screening/sham breathing acid infusion (Porges-stage II) of this study.

In this thesis the sensitising group were found to be generally more anxious, alexithymic, with greater adult attachment vulnerability markers, than the non-sensitising group. This would suggest that the non-sensitising group were emotionally more coherently integrated, enabling them to make more 'emotional' sense of both exteroceptive (psychosocial) and interoceptive (biological) stressors, and were possibly more capable of prefrontal cortex mediated inhibition (e.g. via GABA, oxytocin and vasopressin) of subcortical structures, resulting in subsequent greater adaptive abilities to remain in phase III of the stress and anxiety response for longer (sham breathing), and when called for (stress test) could mount a better (un-impeded) mobilisation (phase I) response, but by means of better mentalisation processes could be able to "self sooth" more effectively resulting in shorter time intervals before returning to the

baseline phase III homeostatic regulation, resulting in less oesophageal pain hypersensitivity. Further research is now needed to address the existing limitations in our understanding of the role of pervasive supra spinal stress regulation in the development of clinical hypersensitisation conditions and to replicate and confirm present findings and posited theories regarding the stress/anxiety regulation as suggested above by using larger cohorts.

7.6 A proposed consilient model incorporating observed ANS stress responses:

Drawing from a number of models discussed in this thesis, one can come closer to a "unity of knowledge", as E.O. Wilson has used the term (6) with regard to the greater implications and impact on our current aetiological understanding of FGID and medically unexplained symptoms. Currently in patients with FGID, visceral pain hypersensitivity (VPH) is thought to be an important mechanism in the development of chronic pain, (43) however the factors that predict the development of chronic pain due to VPH in these patients after inflammation or injury to the GI tract is not well understood. The precise physiological mechanisms for inter-individual differences in the differing degrees of VPH after gut inflammation or injury are difficult to identify. In addition to the severity of the external stressor, factors such as psychological state and trait, genotype, early life experiences and physiological factors such as the biomechanical properties of the gut are all important. To clarify some of these aspects, a proposed consilient view would start with our present FGID conceptualisation of bio-psycho-social, but now to extend it with the addition of the hitherto poorly understood physiological ANS stress

regulatory mechanisms, as suggested by the results obtained in this thesis:

Looking at figure 116, one could start at the 'over-lap' of sociological (Figure 121 - purple, upper-right) with the psychological (Figure 121 - orange, upper-left) domains. The most common precipitating factors are *(A) life events*, (35) with concomitant on-going *(i) psychological* and *(ii) physical stress*; (24) and chronic medical *(B) symptoms*. As clinically it is observed that pain is one of the most common presenting complaints, irrespective of it being explained or unexplained, (89) followed by dysfunction and distress. These two factors *(A&B)* lead to *(C) exteroceptive* and *(D) interoceptive stress* respectively, affecting (purple arrow) the psychological domain of the patient, with its resulting activation of the *"emotional motor system" (EMS)*.

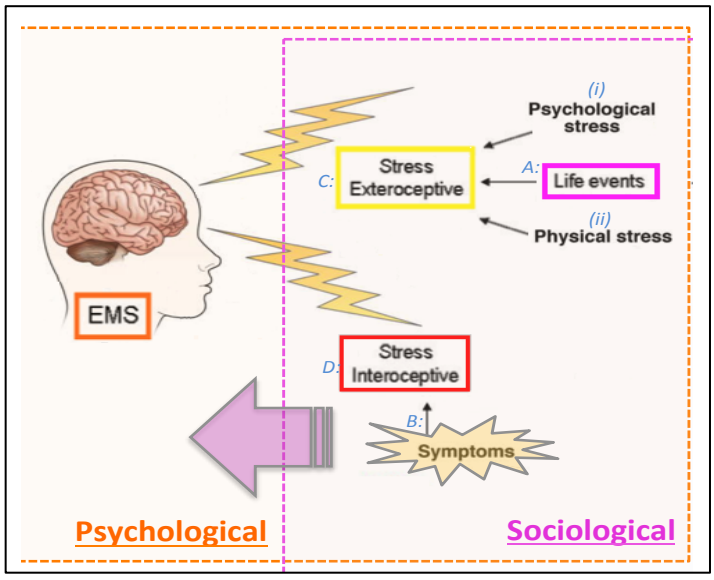


Figure 116 ***A proposed holistic, hierarchical, integrated conceptual-working model, incorporating the dynamic interplay of the observed ANS responses with four domains of centrally sensitised patients. Illustrated here is the effect of the Sociological domain on that of the Psychological. For the full conceptual-model, of which this is a part, see figure 121. (For abbreviations and explanation see accompanying text.)***

Looking at figure 117 (and Figure 121 - orange, upper-left), **Psychologically** predisposing factors of mental state/trait, adult attachment type, and 'mentalisation status' (362) (e.g. degree of alexithymia or "emotional intelligence"(363)) is here of immediate import. This is affected by the patient's *(1) genotype*, and its expression by environmental factors. For instance heritability-twin studies in IBS, found that social learning contributed an equal or even greater influence than genetic heredity alone. (17) Regarding *(2) early life influences*, a co-morbid, or concomitant psychiatric diagnosis, history of abuse (mental/physical) or abandonment/neglect, and previous trauma, are of particular note. These vulnerability factors affect the level of *(3) vigilance* (e.g. hyper-vigilance, anticipatory anxiety & catastrophisation), which affect the 'cognitive-evaluative', and degree of *(4) arousal* (emotional valance) - 'affective-motivational' dimensions of the pain neuromatrix as proposed by Melzac *et al.* (77, 78), and hence causing greater activation of the *(5) "emotional motor system" (EMS)* as proposed by Drossman *et al.* (109). This then induces more involvement from the subcortical structures e.g. *(6) amygdala* (mediating emotions),(122) *(7) periaqueductal grey (PAG)* (mediating defence: avidness/approach response behaviours), *(8) hypothalamus (homeostasis)* and *(9) facial/laryngeal- "visceral" responses* (99, 100)(mediating inter-personal communication). It is at the activation of the sub-cortical level that the psychological aspect eventually affects the brainstem structures (orange arrow), which then triggers the ANS response of the physiological domain.

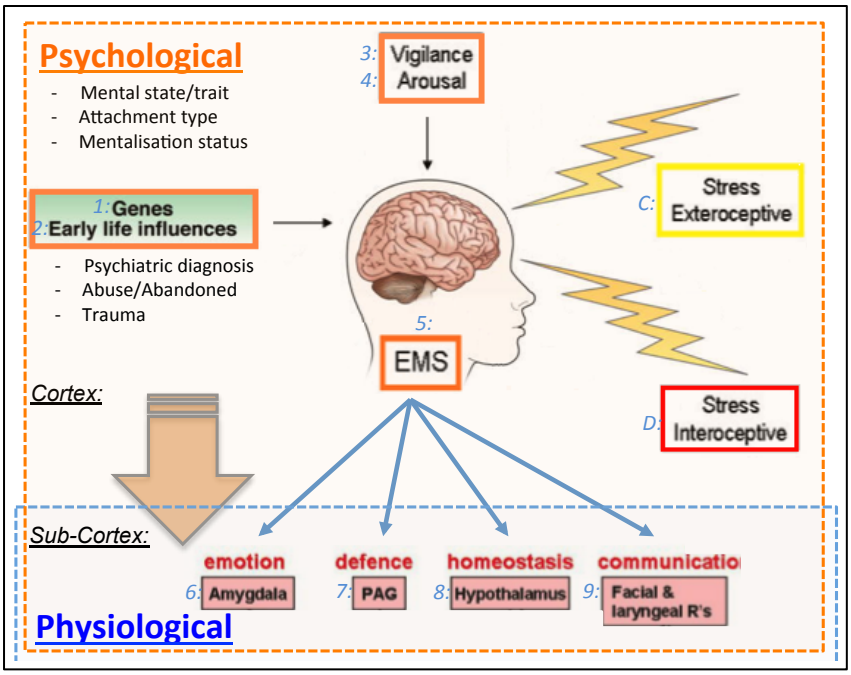


Figure 117 ***A proposed conceptual-working model, incorporating the dynamic interplay of the observed ANS responses with four domains of centrally sensitised patients. Illustrated here is the effect of the Psychological domain on that of the Physiological. For the full conceptual-model, of which this is a part, see figure 7.8. (For abbreviations and explanation see accompanying text.)***

Now considering the involvement of the **Physiological** ANS response domain (Figure 118 and figure 119 - blue bottom left), the basic Cardio Vagal Control (CVC) reflex cycle as proposed by Julu *et al.* (219) needs first to be illustrated. The *(10) Nucleus Tractus Solitarius (NTS)* (green triangle) regulates the cardio vagal motor (CVM) centre (purple half-moon) that also has efferent nerve inputs from the *(12) Nucleus Ambiguus (NA)* that innervate the sinoatrial node of the heart in the modulation of the heart rate (HR). This then has a regulatory feedback loop, via changes in the blood pressure (BP) on the baroreceptors which then connect back to the NTS, and influences the beat-to-beat fluctuations as seen by monitoring heart rate variability (HRV).(10) To complicate things, CVC's control at this juncture is also regulated by the opposing effects of the *(13) SNS* spinal cord afferents, but further augmented by the control exerted by the unmyelinated nerve fibres

from the *(14) Dorsal Motor Nucleus (DMNX)* of the vagus. The CVC/SNS's regulatory effect on the HR is thus best understood across a spectrum of responses, with differing degrees of activation from *(12) NA*, *(13) SNS* and the *(14) DMNX*, and this complicated interplay is best understood by the 'Dynamic Systems Approach' (2-D & 3-D) – autonomic space conceptualisation as proposed by Berntson *et al.*(139) (See chapter 1, section 1.9.3, page 51) Finally the ANS response works in concert with the *(11) HPA/Immune - response*, (not here discussed) giving rise to the patient's eventual clinical presentation by a multiplicity of means, but of particular note for this model is that of sensory modulation, via the regulation of spinal dorsal horn neurones, activating a number of receptors, including NMDA receptors, resulting in the development of central sensitisation, that affects the degree of nociception.

With this understood, one can see how the psychological domain via the activation of the subcortical structures innervate the brain stem ANS response. It is here that the observations as discussed with figures 114 & 115 come into effect. Depending on the psychological environmental threat assessment, one will find differing variations of PNS/SNS activation as proposed by Porges *et al.*(8, 100, 118). In a 'Safe' environmental threat assessment, the main regulation is via the *(12)* NA (III), during 'Un-safe' assessments, the *(13)* SNS (II) is increasingly more activated with a coinciding withdrawal of NA activity, enabling "fight-or-flight" in the short term, and increase in anxiety in the long. When deemed to be in a 'Life-threatening' situation, the *(14)* DMNX (I) co-activation increases, to facilitate the "deer in the headlights" freeze response in acute situations, but an impeding 'avoidant-denial' type procrastination behaviour in the long term. This implies that in the majority of FGID cases there would be an abnormal autonomic substrate, but merely as a part of the mechanism of the underlining disorder, and not as a primary aetiology.

This then through sensory modulation, has a bearing on the persons *(15) Sensitisation status*, which then can develop to have clinical significance (blue arrow).

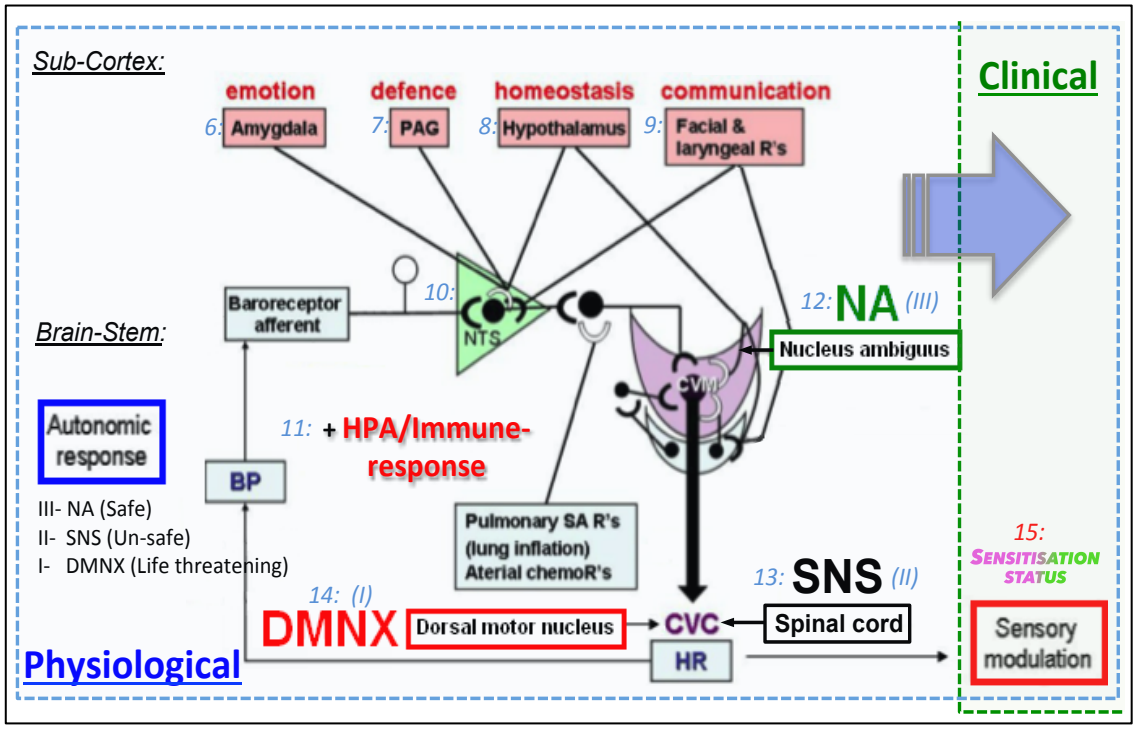


Figure 118 ***A proposed conceptual-working model, incorporating the dynamic interplay of the observed ANS responses with four domains of centrally sensitised patients. Illustrated here is the effect of the Physiological domain on that of the Clinical. For the full conceptual-model, of which this is a part, see figure 7.8. (For abbreviations and explanation see accompanying text.)***

In considering the **Clinical** (Bio) domain (Figure 119 and figure 121 - green, bottom right), as mentioned above the sensory modulation affects the *(15) Sensitisation status*, that explains the physiological mechanism for the observed inter-individual differences in the differing degrees of *(16) Visceral Pain Hypersensitivity (VPH)* that is clinically observed by the *(17) GI pathophysiological symptoms*. *(16) VPH* contributes towards the *(17) GI pathophysiological symptoms* observed, giving rise to pain, dysfunction and patient distress. (43) Due to resulting *(18) alteration in GI function*, medical consultation, and specialist

referrals ensue, which is followed inevitably by investigations and special (more invasive/expensive) investigations e.g. colonoscopy; gastroscopy etc. and finally medications are started. This can have a profound effect on patients, especially when more sinister diagnoses like neoplasms need to be excluded, affecting the patient's social domain (green arrow).

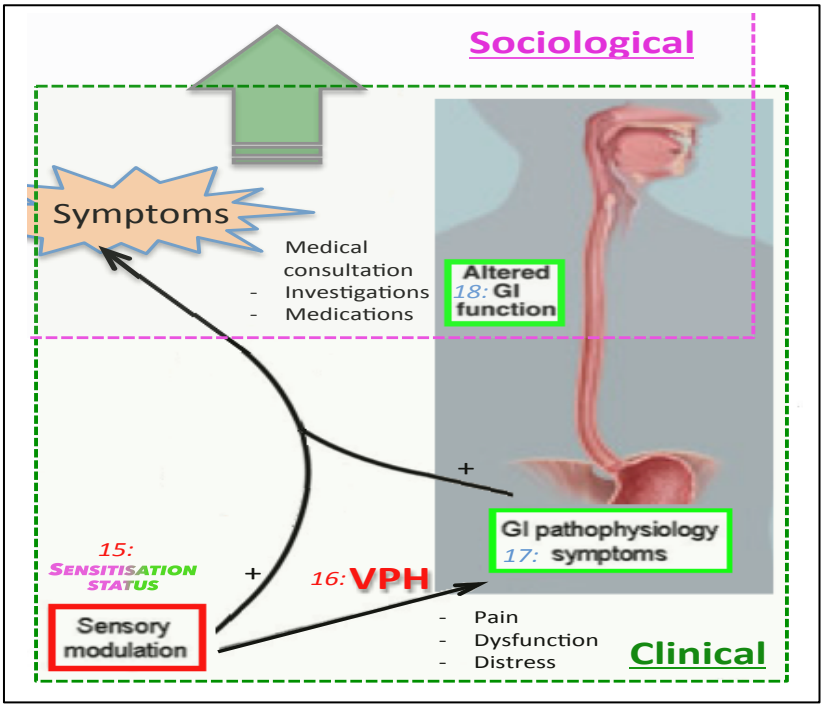


Figure 119 *A proposed conceptual-working model, incorporating the dynamic interplay of the observed ANS responses with four domains of centrally sensitised patients. Illustrated here is the effect of the Clinical domain on that of the Sociological. For the full conceptual-model, of which this is a part, see figure 7.8. (For abbreviations and explanation see accompanying text.)*

Finally in considering the **Sociological** (Figure 120 and figure 121 - purple, upper-right) domain, the effect of chronic medical symptoms is seen in poor quality of life (QoL), (14) commonly due to sleep disturbance, low energy/libido, social withdrawal and varying degrees of anhedonia and dyshedonia. (45) As a result of this in many cases there are accounts of increased sick leave (poor productivity), with increasing 'tension/stress' at the work place because of this. In extreme instances this leads to the

complicated process of 'dismissal on medical grounds' and disheartening negotiations associated with the turbulent process of securing state funded 'Disability Living Allowance' and its accompanying stigma. 'Learnt helplessness' and deskilling externalisation of the individual's locus of control is also seen in cases, (14) which then contribute to poor interpersonal relationships, with increased strain and dependence on the individuals' families and/or caregivers ('carers fatigue'), which due to mounting desperation can lead to an increase in consultation behaviour, and resulting poor doctor-patient relations. (15) Patients' *(19) adaptive or maladaptive behaviours* are influenced to a great extent by the actual, or perceived sociological environment which can range from 'safe' to 'life threatening', determining in turn how they cope with and deal with inevitable *(A) life events* (35) and its subsequent demand on the psychological domain (purple arrow), bringing one full circle to where the discussion began.

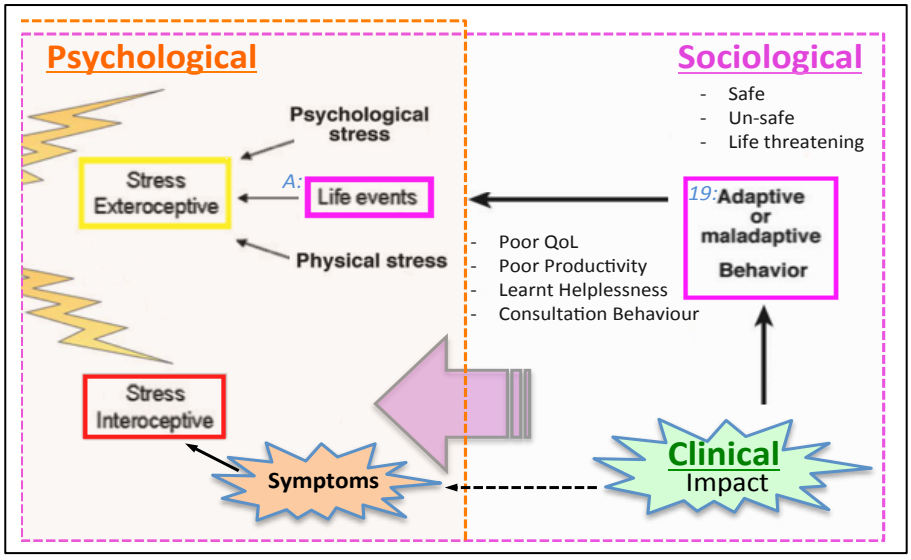


Figure 120 ***A proposed conceptual-working model, incorporating the dynamic interplay of the observed ANS responses with four domains of centrally sensitised patients. Illustrated here is the effect of the Sociological domain on that of the Psychological. For the full conceptual-model, of which this is a part, see figure 7.8. (For abbreviations and explanation see accompanying text.)***

A consilient view would entail the "weaving together" of all the constituent parts and their respective models as highlighted throughout the discussion, into one united perspective as illustrated in figure 121 below. It is then that (i) the ANS responses observed's true context and impact can fully be appreciated and (ii) the circular re-enforcing nature of the interactions becomes evident. The circular re-enforcement is of particular note, as over time its spiralling course can produce the full complement of the chronic/perpetuating biopsychosocial factors observed in some of the extreme "heart-sink"- or -"revolving-door" VPH patients, that can place a considerable burden on personal, professional, financial and even national resources. (16) Finally, it could assist in the earlier 'pro-active' identification of individual key areas that could be specifically targeted. This could potentially reduce inappropriate referrals and guide more timely clinically relevant referrals and cost effective multidisciplinary interventions.

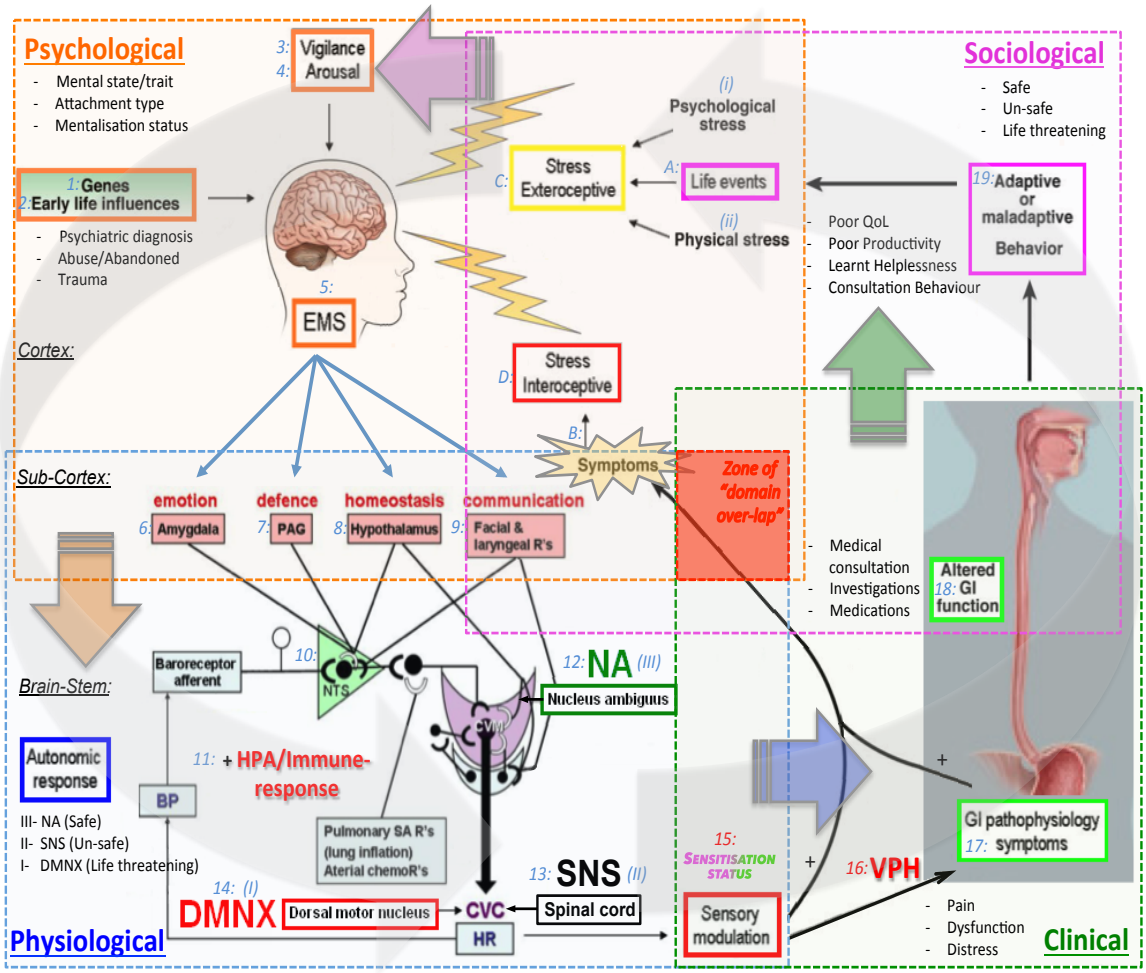


Figure 121 *A new holistic, hierarchical, integrated conceptual-working model, incorporating the dynamic interplay, of four domains; Psychological, Physiological, Clinical & Sociological; along with the incorporation of the novel physiological (ANS) regulatory mechanisms as observed in this study. The interplay of factors affecting patients with chronic visceral pain hypersensitivity (VPH) disorders, as seen in clinical practise can be better understood, as in extreme cases they can exhibit the 'full compliment' of the biopsychosocial triumvirate. (For abbreviations and explanation see accompanying text.)*

7.7 Implications on therapeutic approaches

7.7.1 Psychopharmacology

Despite laudable progress in gastrointestinal neuroscience research, directed towards describing the culpable mechanisms that account for development of visceral pain, in conjunction with considerable

investment in drug development, translation into tangible pharmacological improvements in patient outcomes have remained poor. (51, 338) Moreover, given that the contemporary pharmacological armamentarium has limited efficacy, and in some cases marked concerns regarding safety, (339) it comes as no surprise that the multidisciplinary approach utilising a number of psychosocial and psychophysiological treatments has been used in the treatment of visceral pain. (340, 341) However it is already common practice for antidepressants to be used for chronic functional pain disorders. The circular observations of visceral pain inducing dyshedonia, and the response of negative emotional context e.g. stress and anxiety enhancing the visceral hyperalgesia induced provides rationale for how antidepressant therapy works. This is relevant for both pharmacological and psychological therapies. Because there is a clinical tendency to focus on more typical "anti-nociceptive" treatments in functional syndromes, whereas evidence suggests that co-morbid emotional problems are under diagnosed and undertreated, (364, 365) which if left untreated may then lead to less effective treatment responses and outcomes. The implication suggested by this thesis is that rather than treating either pain or emotion, *both* need to be simultaneously addressed, as they are mutually re-enforcing.

7.7.2 Psychotherapy

The results from this thesis would suggest the incorporation of the behavioural intervention of "paced deep breathing" as part of a therapeutic 'package' aimed at patients where VPH is suspected. In study 4 (the placebo controlled /atropine challenge study), it was observed that in both arms of the study there was modulation of the induced sensitisation and hyperalgesia suggesting that the non-specific (psychological/placebo-effect) component of deep breathing

produces analgesia that can modulate spinal nociception and thus influence the development of central sensitisation and hyperalgesia. (366) This may relate to activity in supraspinal pain modulatory systems involving opioids. (367) The behavioural interpersonal interaction may be activating these systems by the higher cognitive factors of the intervention such as distraction, expectation and anticipation, transmitting its effects 'downstream' to the spinal dorsal horns to have an impact on the development of central sensitisation through the modulation of chemical signalling and receptor function. The fact that acid-induced sensitisation can be modulated by the non-specific (psychological/placebo-effect) component of deep breathing confirms that supraspinal mechanisms are important and should also be incorporated in treatment interventions modulating pain sensitivity.

However in study 4 the 'paced deep breathing' demonstrated efficacy over and above that of the atropine's 'knock-out' arm in spite of also receiving the nonspecific therapeutic elements associated as above, by being "paced". This strongly suggests that the increased CVT tone *per se* adds additional analgesia in central sensitisation induced VPH, and thus could provide additional 'direct' clinical efficacy in symptom reduction. The intervention could still continue to offer the more traditional 'indirect' therapeutic use of deep breathing in reducing levels of subcortical and visceral arousal, by means of the so-called "calming breath" (368) and induction of the "relaxation response". (369) The results of studies 2 and 4 could also be applied clinically by using the deep breathing intervention in patients undergoing biofeedback training for pain-related diseases. Deep breathing techniques may also be used in a variety of chronic pain states, which are characterised by clear limitations in response to drug treatment, and can be tailored to the individual needs of each patient. Furthermore, since the modulation is physiological rather than

pharmacological, the treatment is not associated with any negative health side effects.

If this is combined with mindfulness (370) and mentalisation strategies, (362, 371) the alexithymia, attachment vulnerability and its associated misappropriated somatic tension and subsequent activation of the EMS, indicated by this thesis, could also be addressed. With regard to the attachment vulnerability, more therapeutic skill would be required however, as it would suggest the facilitation of the patients' ability to interpret their 'intrinsic somatic-emotions' by developing a coherent extrinsic life narrative, that incorporates a prefrontal mentalisation[22] of the subcortical/brainstem arousal patterns/symptoms, and its resulting interpersonal implications. Dan Siegel coined the term 'mindsight' to describe this ability, and states, originally with regard to better parenting, but applying equally to pain management, the following:

> *"A coherent life story is one in which the adult has made sense of his or her own childhood experience […or visceral pain symptoms] and has insights into how that past has influenced his or her development as an adult and as a parent […or patient]. Making sense is revealed in a flexible and reflective narrative that is predictive of that adult's child having a secure attachment, […or being able to demonstrate better cortical pain regulation in chronic sensitising pain conditions]."*(373)

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[22] **Mentalisation**; defined generally as: *(i)"To make mental in nature, rather than physical", or in psychology as: (ii) "To understand the behaviour of others as a product of their mental state."*

This mentalisation aspect combined with CBT (374), interpersonal (375) or hypnotherapeutic (376, 377) interventions in conjunction with the behavioural 'paced deep breathing' component would seem to be addressing the '*area of overlap*' in the four domains as referred to in the proposed conceptual model above, suggesting the area of 'most return'. (Figure 117) This is where with the least amount of resource input, applied to the most relevant clinical areas of this specific patient group, could potentially deliver the most cost effective clinical outcomes, as illustrated in figure 122. This hypothesis needs to be developed and tested with more clinically based research.

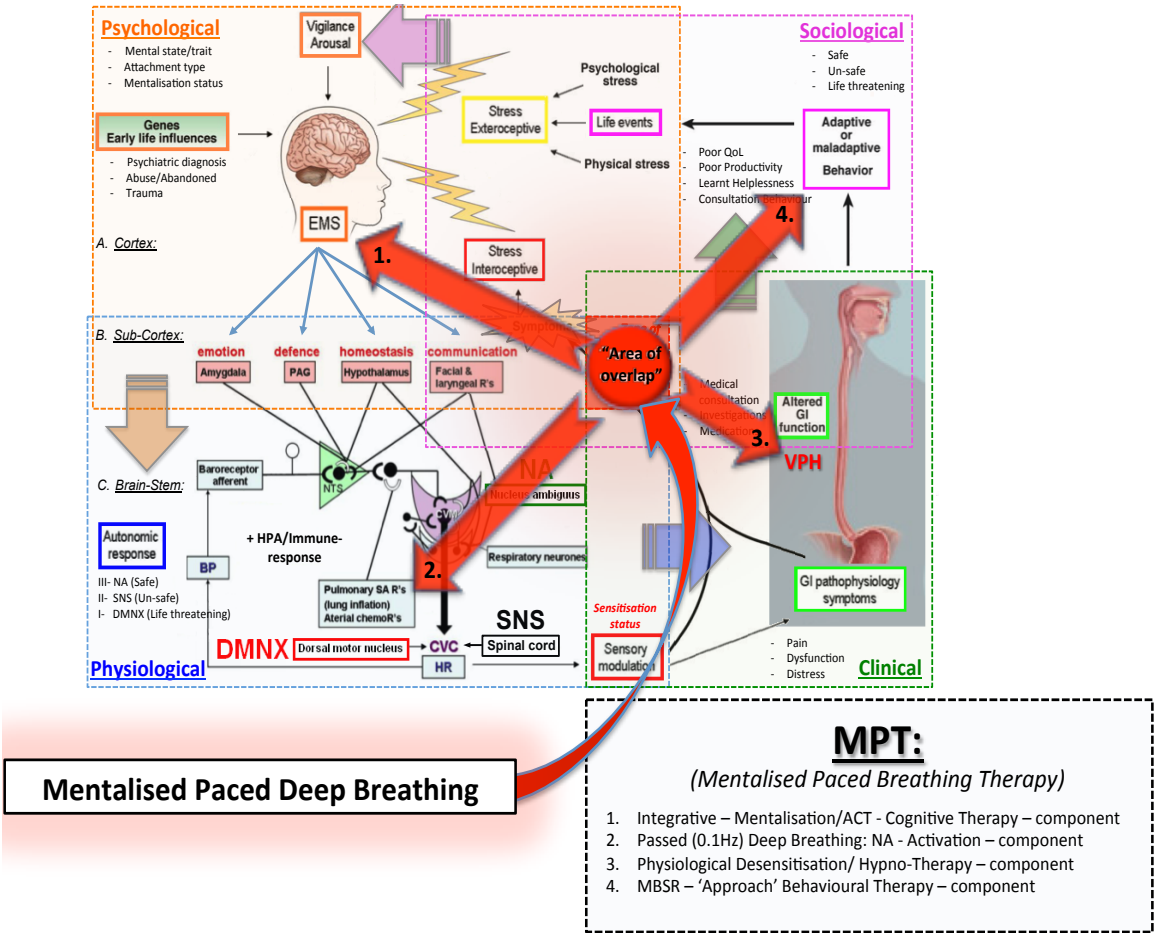

Figure 122 ***Illustrated is an example of a possible new proposed psychotherapy treatment, as suggested by this thesis: "Mentalised Paced-Breathing Therapy" (MPT), incorporating four distinct psychotherapeutic modalities, as components to a therapeutic 'package' specifically designed to affect the "area of overlap" of the psychological, physiological, clinical & sociological domains; as seen in patients with chronic visceral pain hypersensitivity disorders.***

7.8 Limitations of the oesophageal model

The amount of practical, established models of injury-induced visceral hyperalgesia in humans is limited. Amongst them, the acid induction model of OPH, which I used in these studies, is particularly well validated. Having been used now in several significant studies' by a variety of independent research groups, considerable experience and data have been accumulated with this model. It has provided insights into the mechanisms of central sensitisation and the pathophysiology of pain hypersensitivity in a number of different FGIDs, e.g. NCCP. There are inevitably limitations to any model, no matter how validated, and this one is not an exception. These limitations should be appreciated before the extrapolation of conclusions to patient populations.

7.8.1 Nature of the sensitising stimulus

Experimentally, the model consists of a 30-minute infusion of 0.15M HCl in the distal oesophagus, which then induces secondary hyperalgesia in the non-acid exposed proximal oesophagus. Short-lived pain hypersensitivity is produced, with a mean normalisation of nociception within 8 hours. (177) This is most likely due to the acute stimulation of oesophageal nociceptors such as TRPV1 and ASIC, due to the action of the acid giving rise to subsequent induction of spinal central sensitisation, which then affects the observed hyperalgesia. With the completion of the acid infusion, the noxious stimulus and peripheral drive is removed, thus allowing receptor function and the nociceptive processing to normalise.

Clinically however, pain hypersensitivity is often a persistent finding in patients with functional gastrointestinal disorders. In patients with PI-IBS for example, hyperalgesia persists long after the resolution of a previous

infectious insult, most likely due to the nociceptive circuits remaining active. In previous studies using this model of OPH, gastroscopies were performed within 12 hours of experimental acid infusion, (179) which did not shown histological evidence of inflammation. Although increased cytokine production and receptor translation at the infusion site cannot be ruled out, the processes that would be clinically induced in chronic gastro-oesophageal reflux disease is most likely to be significantly different to those seen in subjects.

Thus, the model's short duration of induced hyperalgesia, together with the lack of any identifiable inflammatory response, would suggest it being more reflective of an acute form of "activity dependent" central sensitisation. Clinically this form of sensitisation is initially of significance in the development of visceral pain hypersensitivity, and hence this model is limited in providing information on the mediators of chronic changes in synaptic plasticity following injury. Accordingly the relevance of this model of OPH has limitations when applied to states characterised by long-term post-injury pain hypersensitivity. As it would clearly be unethical to develop a model of chronic post-injury pain hypersensitivity in humans, this problem deserves further consideration.

As chapters 3 to 6 showed that acid infusion *per se* was associated with increased unpleasantness and anxiety scores, the aversive nature of the acid infusion needs also to be considered, as it raises the possibility that any sensory or autonomic changes observed might be significantly driven by psychological factors. Generalised hypervigilance as being the mechanism of observed visceral hyperalgesia can be ruled out, as the lack of change in pain threshold on the foot after oesophageal acid argues convincingly against this. Selective hypervigilance to visceral stimulation however, cannot be completely disqualified. Previous studies

with proximal oesophageal evoked potentials following distal oesophageal acid infusion have shown a decrease in latency of the early components (related to sensory discrimination) with no change in the late components (related to cognitive evaluative factors e.g. vigilance), (378) which would argue against visceral hypervigilance being the mechanism of acid-induced hyperalgesia in the model.

7.8.2 Nature of the pain stimulus

Oesophageal pain threshold testing in the model is achieved by means of electrical stimulation. This modality offers practical beneficial characteristics that include: *(i)* ease of administration, *(ii)* reproducibility *(iii)* well-defined onset and offset, and *(iv)* an ensured short latency to afferent fibre stimulation. (179) On the other hand, a potential criticism of this modality is that it is not as physiological as other modalities in use, like for instance mechanical distension or thermal stimulation.

A second negative characteristic of electrical stimulation is that it directly depolarises all classes of primary afferents and hence bypasses any peripheral receptor mediated transduction, and thus the activity in specific nociceptors cannot be inferred. (51) This said, the modality remains effective in assessing the contributions of mediators to spinal pain processing, allowing for clear information with regard to central sensitisation mechanisms. As other modalities may be associated with different psychological and/or autonomic effects, it would be prudent in future research to replicate some of the findings in this study with multimodal oesophageal stimulation. Studies of this kind might provide more clinically relevant characterisation of oesophageal sensory processing at baseline and after acid sensitisation.

7.8.3 Reproducibility, Carryover and Period effect of the model

Although previous studies with the model (53, 177) did not show significant intra-study change in the degree of sensitisation to suggest an order effect and as such demonstrated good repeatability of the magnitude of acid-induced sensitisation within studies. The data presented in this thesis has however reproduced results found on at least one occasion using this model. (179) This would suggest that when sensitisation is examined across studies, i.e. involving longer periods of time with the same subject cohort (more interval studies), the degree of acid-induced hyperalgesia might diminish on subsequent studies.

The mechanism of this habituation is unknown but could relate to a variety of peripheral and central factors. Neurologically, receptor desensitisation – and – down-regulation, increased descending spinal pain inhibition, and reduced pain facilitation may reduce neuronal-sensitivity responses on repeated study. Psychologically, there is a gradual reduction of the dopaminergic attenuated 'novelty response' that affects the quality of subject attention. Due to anxiety habituation brought about through repeated behavioural exposure the subjects' emotional valance is also gradually diminished. Finally, with repeated visits due to familiarity, unavoidable changes are facilitated in the interpersonal response between research staff and subjects that could also have an increasing longitudinal effect.

During the design of the studies in this thesis, the potential of the carry-over effect was debated in some detail and it was decided that a period of at least 2 weeks would address this concern from a standpoint of pragmatism. (379) The meantime between visits was 3 weeks 4 days (range 2 weeks 1 day – 4 weeks 5 days) for studies 2 and 4 and therefore the likelihood of any carry-over effect is small. As is highlighted in the

paper by Mills *et al*. there is an argument that the carry-over effects of interventions across periods as carry-over effects are rare and moreover statistical manipulation per se cannot address the impact of a carry-over effect as they are often under-powered. (379-381)

Further it is hence recommended that subjects are not studied regularly, and that sufficient time intervals between studies are scheduled, such that participation in studies remains as far as possible a novel experience. Randomised double-blind cross-over study formats are here important, with possibly novel "unknown examiners" introduced in the later phases of study, that might help to reduce the development of encountered order effects.

7.8.4 Applicability of the Results to Clinical Populations

The studies presented in this thesis have all been performed in healthy volunteers, and thus from a psychophysiological perspective it is a prerequisite to draw attention to the likelihood of significant differences between these individuals and patients with functional gastrointestinal disorders. Additionally, there may be further psychological variation between volunteers and healthy individuals who do not seek study participation. Factors such as anxiety, past experience and openness to new experiences may be relevant. Further the model used to explore oesophageal sensitivity in these studies is of an acute nature, yet the illnesses seen clinically are chronic. Necessarily in using this model, research findings' direct applicability to clinical populations are informative, but remains speculative and should be done judiciously.

On the other hand, the oesophageal acid model has been extensively validated as a reproducible model of central sensitisation in healthy

individuals, and has already shown clinical validity in some conditions. For example in patients with NCCP, using this model, exaggerated hyperalgesia to acid infusion was demonstrated. (175, 178) Additional factors that could also be observed during these studies, were accompanying reductions in latency of the early oesophageal evoked potential components; suggestive of heightened central sensory processing in these patients. As such, the model has some relevance to patients with states characterised by oesophageal hypersensitivity and can provide useful information, especially in the development of acute post acid injury induced central sensitisation. A possible mechanism proposed by Sharma, for the development of oesophageal hypersensitivity in these patients, could be:

> "*… that repeated episodes of acid reflux induce peripheral and central sensitisation; the latter may then persist in susceptible individuals despite removal of the acid stimulus by appropriate [pharmacological] therapies. Subsequent small volumes or sub-clinical acid exposure may then be sufficient to maintain sensitisation and result in sensory dysfunction manifesting as hyperalgesia and allodynia.*"(179)

Finally, the research in this thesis doesn't directly provide an explanation for symptoms or hypersensitivity in the group of FGID that is preceded by infection or inflammation. In these cases it would focus only on the hypersensitivity component of these conditions, which is found in post infective hypersensitivity as well as in hypersensitivity *de novo*.

7.8.5 Validity and Reproducibility of CVT and the Neuroscope

Central to the findings of this thesis is the validity and reliability of cardiac vagal tone (CVT) and the instrument/method by which it was measured. Due to the methodological shortcomings of the traditionally established

techniques of measuring autonomic nervous system tone (see 2.12, page 96), a commercially available biosignals acquisition system known as a "Neuroscope" was used to measure and record cardio vagal tone (CVT) in this thesis. The Neuroscope is unique in using a process called "phase demodulation" to derive at a measure of cardiac vagal control (CVC), and hence to calculate CVT. (226) It is measured in standardised units on a Linear Vagal Scale (LVS), where 0 was derived from fully atropinised healthy human volunteers. (219) CVT has been demonstrated to both a sensitive and specific measure of vagal tone, comparable to other indices derived from analysis of heart rate variability, (10) and has also been demonstrated to be a reproducible measure of parasympathetic nervous system tone over a period of 1 year. (382) The measurement of cardiac vagal tone has been increasingly utilised as a research tool for deriving PNS tone across a diverse range of research themes. (271, 311, 383)

The technique and concept of CVT is of interest but not without conceptual concerns, particularly as there is a paucity of clinical data evaluating patients with known dysfunction of the autonomic nervous system using this technique. Similarly to HRV measures, this technique does not actually measure vagal tone *per se* but high-frequency modulation of HR. Thus it is based on an identical physiological concept as HRV, resting upon the assumption that the short latency of HR responses to blood pressure changes reflects the vagal limb of the baroreflex arc, in contrast to the longer latency of HR responses due to sympathetic activation/deactivation.

Moreover, a continued conceptual concern of all techniques using heart rate variability as a surrogate marker of autonomic tone are, by definition, derived from cardiotropic parameters. Therefore, their direct

applicability and correlation to the autonomic tone occurring at the proximal and mid regional and mucosal level of the GI tract is largely unknown and warrants further systematic investigation. For instance, it would be interesting to concomitantly measure HRV parameters of vagal tone in association with other objective physiological markers in the gut. For instance one could measure pancreatic polypeptide as its excretion is exclusively under vagal control. In addition, it would also be possible to measure transient relaxations of the lower oesophageal sphincter as these occur as a sequelae of the vago-vagal reflex. Nevertheless, whilst these studies remain to be performed, cardiotropic measures do offer a potential insight, until further refinement via future technologies.

7.8.6 Study-design and Statistical limitations

This thesis explores by using a randomised crossover trial design to derive the main hypothesis testing study findings. The study design and the choice of statistical analysis used could have some methodological limitations that deserve attention. Of note is the use of statistical methods for repeat measures in the crossover analyses (i.e. liner mixed models), as opposed to the preferred intention to treat analyses, where all patients randomised are analysed, despite the intervention they actually received. The potential shortcoming could be that the '*per-protocol*' analyses may bias the results and may be seen to be used only for the rationale that the latter reveals statistical significance and thus of interest to be reported; whereas an additional intention to treat analyses would be possibly more appropriate to improve scientific rigor. (384-386) Convention would encourage presenting the results with non-sensitisers and, if not done, to justify the rationale and to illustrate what the results would be if non-sensitisers were included and how the lack of data on non-sensitisers could have influenced the results.

Even though a "*per protocol* analysis" could potentially bias the results and notwithstanding the fact that this is of paramount importance in crossover trials as highlighted in the literature, (384-386) however, these principles are not directly applicable to the hypothesis testing studies in this thesis. If one considers the relevant studies in turn:

Study 2 - The study design was such that sensitisation/non-sensitisation was defined on the results obtained from the study visit in which the participants were exposed to the 'sham breathing' arm. If participants were randomised to the sham breathing intervention during the first visit of study 2, they were excluded and did not attend study 2's second visit where deep breathing would have been studied (see figure 69, page 206). Conversely, subjects who were randomised to the deep breathing intervention during the first visit, and underwent the deep breathing protocol during the second visit, could potentially have been included in an "intention to treat" analysis. However, it was actively chosen not to include this data for the following reasons:

1. As not all non-sensitisers were exposed to both visits, it was deemed not appropriate to present an analysis on a proportion of these participants, as this would introduce an element of bias in the analysis *de novo*.
2. Non-sensitisers were defined as having no reduction, or a reduction of <6 mA in proximal oesophageal PT, after acidification and therefore such subjects do not display secondary hyperalgesia in response to the acid infusion model. Thus, the anti-hyperalgesic/analgesic effect of deep breathing cannot be assessed in this subgroup as they do not sensitise to the stimulus.
3. If one had included this subgroup in our analysis, it may have conversely over-estimated the effect of 'deep breathing' and 'sham breathing' as these participants did not sensitise.

Study 4 – In study 4, there was a screening visit, after which the non-sensitising participants were excluded and therefore these subjects were not exposed to the 'deep breathing' or atropine treatment arm. The design of this study included a screening visit as it was judged inappropriate and unethical to expose healthy participants to intra-venous atropine if they were going to be excluded. Hence an "intention to treat" analysis would also be inappropriate for similar reasons as stated above. Future studies will have to account for this with improved designs.

7.9 Future Directions

In this thesis I concentrated on using atropine as a muscarinic antagonist to enhance visceral pain perception. A future line of enquiry using pharmacological agents would be to focus on muscarinic agonists, to increase the PMS activity. Alternatively the focus should be on reducing the SNS activity by means of modulating catecholamine function. Here Alpha 2 agonists: (clonidine) stimulate presynaptic alpha 2 receptors to mediate feedback inhibition of noradrenaline release. Postsynaptic alpha 2 receptors in the vicinity of the NTS and rostroventrolateral medulla are important determinants of sympathetic outflow. Clonidine acts on these receptors to significantly reduce sympathetic outflow to the cardiovascular system to cause hypotension and bradycardia. On the other hand, Beta agonists: Stimulation of both beta 1 and 2 receptors (isoproterenol) increase cardiac contractility, heart rate and cardiac output. Beta antagonists: Propanolol is a competitive inhibitor of sympathomimetic amines at both beta 1 and beta 2 receptors and therefore counteracts the effects of isoprenaline. It reduces heart rate, contractility and blood pressure.

Hence clonidine and beta-blockers has the potential of acting like pharmacological agents producing similar effects to "paste deep breathing". Of these clonidine offers the most potential, because of its ease of administration, high bioavailability and for its future potential therapeutic role. A proposed study could be to use clonidine in this model of acid induced VPH, where clonidine will be administered orally at a single dose of 5.5 micrograms/kg. Side effects should be monitored and the subjects could be given cognitive tasks to perform to control for the sedative effects of clonidine.

Further, the factors regarding the healthy volunteers who in spite of repeated acid infusion and stress modulation still failed to sensitise, remains unclear. Although some light was shed on possible aspects associated with this group in study 3, as such it represents a beginning, but more experimentation using this model is still necessary to fully understand their phenotype. Potential research here would be to repeat acid exposure studies, but this time using a different more effective psychological stressor. To date strategies recalling past sad life events, (179) as well as exposing subjects to pictures of different emotive faces at time of acid exposure, have been proven constructive and could offer better opportunities of eliciting more explicit physiological results. (268) A second line of enquiry could be to antagonise their endogenous opioid systems with naloxone prior to acid infusion in order to determine whether sensitisation could then be induced.

The clinical applicability of the results in this thesis remains to be explored. The findings of studies 2 and 4 confirmed that autonomic control plays a prominent role in the development of central sensitisation and that by increasing vagal tone, induced visceral pain hypersensitivity can effectively be reversed. These results are encouraging, but remain

speculative, as they represent laboratory findings of an acute exposure in a healthy volunteer group. Of importance is now to replicate these outcomes in patients with chronic visceral pain syndromes. A suggested initial study here would be, by using a marker of oesophageal sensitivity, to compare the introduction of 'paced breathing' in patients in a laboratory setting. If these findings remain positive, a further clinical study incorporating the 'paced breathing', as a component of a specially designed treatment strategy in actual patients should be undertaken. A potential study that needs to be concluded in the clinical setting would be a three tiered randomised control trial, whereby the 'paced breathing treatment intervention', is compared to a 'placebo control' using similar psycho-education, but replacing the breathing-component with a standard 'relaxation intervention', and the medical treatment as usual. A second important clinical study would be a 'head-to-head' comparison study of the 'paced breathing treatment intervention' with an intervention with proven efficacy like for instance hypnotherapy. The impact and cost-effectiveness of treatment strategies should then be assessed and compared.

The literature supports the notion that psychophysiological factors e.g. trait anxiety and neuroticism, influence an individual's visceral pain sensitivity and tendency to develop post-injury OPH. This thesis goes on to imply that these factors are also influenced by alexithymia and attachment vulnerability. The data presented would suggest that an awareness of these issues might assist in more effective patient management, as it has the potential to identify likely patients at higher risk of developing chronic pain in individuals suffering with acute visceral pain. It could also assist distinguishing between patients who might cope poorly with chronic pain, and those who may have difficulty in engaging with healthcare services, affecting prognosis and potential healthcare

costs. Thus, regarding this issue further powered studies are also required to confirm the novel but putative observations concerning the alexithymia/attachment association with central sensitisation and in OPH patients. A potential study that could clarify this would be an epidemiological based cohort study. Here all first attender patients at a designated specialist gastroenterology clinic would undergo routine alexithymia/attachment profiling, with subsequent outcome follow-up relating to diagnosis, treatment and prognosis. A correlation between trait vulnerability and outcome could then be highlighted and its clinical presence confirmed. A similar study could also be done in a match control group in a related medical/surgical clinic to probe if potential correlations are specific FGIDs or a general medical finding.

Finally, regarding genetic profiling, it will suffice to say here that it will always be a high priority in pain research and all opportunities to possibly identify genetic factors should be pursued.

7.10 Conclusions

The studies in this thesis have investigated the psychophysiological modulation of autonomic responses involved in acid-induced oesophageal pain hypersensitivity.

My studies suggest that an important role is played by the autonomic control in the development of central sensitisation, and demonstrated that by increasing PNS tone, induced visceral pain hypersensitivity can effectively be reversed. In addition it also demonstrated that the processes that determine the development and magnitude of post-injury pain hypersensitivity in the human viscera are complex and intertwined. Cognitive factors such as anxiety, alexithymia and attachment status

influence supraspinal mechanisms and autonomic responses, which in turn modulate the development and degree of central sensitisation in the viscera. Certain individuals may also be predisposed to greater injury-induced sensitisation in the viscera based on their psychophysiological and genetic profiles.

The development of effective therapies for patients with FGID requires the clear understanding of the relevant psycho-pathophysiological processes involved and their modulating interactions. Due to the complexity of these processes the incorporation of effective characterisation of at risk phenotypes and development of specifically tailored treatment strategies, may be the key to developing targeted, effective and cost-effective therapeutic agents in patients with chronic visceral pain syndromes.

Hence in conclusion, the modern physician, just like in the time of Hippocrates, may once again make little distinction between emotional and physical wellbeing, (2) as physical and mental concepts are once again being interchanged and found to be equally relevant at procuring diagnosis and cure; but now with greatly increased awareness and respect for the complex-intertwined nature of the 'body/mind'. (3)

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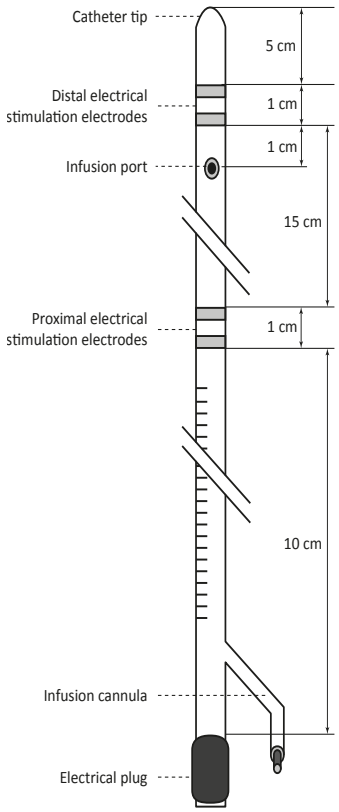
Appendix One

Additional Technical Specifications

1. Bespoke naso-oesophageal catheter



The bespoke

UniTip™

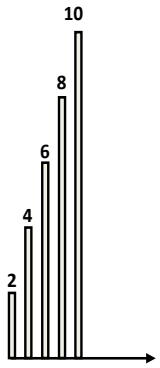
- naso-oesophageal catheter

(Katheter 6F, UNISENSOR AG, Attikon, Switzerland)

2. Dimensions of electrical stimulation paradigm

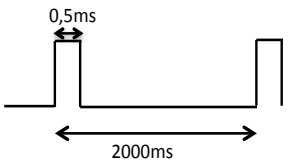
The stimulation paradigm:

Pulse strength: 200V, and
variable mA with incremental increase of 2 mA per pulse.
Pulse width: 500µs
Pulse number: single pulse (no train);
Pulse form: square pulse (not sinus wave);
Pulse frequency : 0.5Hz (1puls/2seconds),
repetition in 'free run' mode (no delay).



Variable mA (200V)
incremental increase
2 mA/pulse

NB: this is done manually by the investigator.



Pulse width: 500µs (square)
Timing: 0.5Hz – 'free run'
(the pulse is generated automatically and continuously.)

Appendix Two

Psychological Assessment Questioners

1. Big Five Inventory (BFI)

BIG FIVE INVENTORY (BFI)

Reference

John, O. P., & Srivastava, S. (1999). The Big-Five trait taxonomy: History, measurement, and theoretical perspectives. In L. A. Pervin & O. P. John (Eds.), *Handbook of personality: Theory and research* (Vol. 2, pp. 102–138). New York: Guilford Press.

Description of Measure:

44-item inventory that measures an individual on the Big Five Factors (dimensions) of personality (Goldberg, 1993). Each of the factors is then further divided into personality facets.

The Big Five Factors are (chart recreated from John & Srivastava, 1999):

Big Five Dimensions	**Facet (and correlated trait adjective)**
Extraversion vs. introversion	Gregariousness (sociable) Assertiveness (forceful) Activity (energetic) Excitement-seeking (adventurous) Positive emotions (enthusiastic) Warmth (outgoing)
Agreeableness vs. antagonism	Trust (forgiving) Straightforwardness (not demanding) Altruism (warm) Compliance (not stubborn) Modesty (not show-off) Tender-mindedness (sympathetic)
Conscientiousness vs. lack of direction	Competence (efficient) Order (organized) Dutifulness (not careless) Achievement striving (thorough) Self-discipline (not lazy) Deliberation (not impulsive)
Neuroticism vs. emotional stability	Anxiety (tense) Angry hostility (irritable) Depression (not contented) Self-consciousness (shy) Impulsiveness (moody) Vulnerability (not self-confident)
Openness vs. closedness to experience	Ideas (curious) Fantasy (imaginative) Aesthetics (artistic) Actions (wide interests) Feelings (excitable) Values (unconventional)

For more information about the Big Five, visit this website:
http://www.uoregon.edu/~sanjay/bigfive.html#where

Abstracts of Selected Related Articles:

Bouchard, T. J. & McGue, M. (2003). Genetic and environmental influences on human psychological differences. *Journal of Neurobiology*, *54*, 4-45.

> Psychological researchers typically distinguish five major domains of individual differences in human behavior: cognitive abilities, personality, social attitudes, psychological interests, and psychopathology (Lubinski, 2000). In this article we: discuss a number of methodological errors commonly found in research on human individual differences; introduce a broad framework for interpreting findings from contemporary behavioral genetic studies; briefly outline the basic quantitative methods used in human behavioral genetic research; review the major criticisms of behavior genetic designs, with particular emphasis on the twin and adoption methods; describe the major or dominant theoretical scheme in each domain; and review behavioral genetic findings in all five domains. We conclude that there is now strong evidence that virtually all individual psychological differences, when reliably measured, are moderately to substantially heritable.

Tkach, C., & Lyubomirsky, S. (2006). How do people pursue happiness?: Relating personality, happiness-increasing strategies, and well-being. *Journal of Happiness Studies, 7*, 183-225.

> Five hundred ethnically diverse undergraduates reported their happiness strategies – that is, activities undertaken to maintain or increase happiness. Factor analysis extracted eight general strategies: Affiliation, Partying, Mental Control, Goal Pursuit, Passive Leisure, Active Leisure, Religion, and Direct Attempts at happiness. According to multiple regression analyses, these strategies accounted for 52% of the variance in self-reported happiness and 16% over and above the variance accounted for by the Big Five personality traits. The strongest unique predictors of current happiness were Mental Control (inversely related), Direct Attempts, Affiliation, Religion, Partying, and Active Leisure. Gender differences suggest that men prefer to engage in Active Leisure and Mental Control, whereas women favor Affiliation, Goal Pursuit, Passive Leisure, and Religion. Relative to Asian and Chicano(a) students, White students preferred using high arousal strategies. Finally, mediation analyses revealed that many associations between individuals' personality and happiness levels are to some extent mediated by the strategies they use to increase their happiness – particularly, by Affiliation, Mental Control, and Direct Attempts.

Shiota, M.N., Keltner, D., & John, O. P. (2006). Positive emotion dispositions differentially associated with Big Five personality and attachment style. *The Journal of Positive Psychology, 1*, 61-71.

> Although theorists have proposed the existence of multiple distinct varieties of positive emotion, dispositional positive affect is typically treated as a unidimensional variable in personality research. We present data elaborating conceptual and empirical differences among seven positive emotion dispositions in their relationships with two core personality constructs, the "Big Five" and adult attachment style. We found that the positive emotion dispositions were differentially associated with self- and peer-rated Extraversion, Conscientiousness, Agreeableness, Openness to Experience, and Neuroticism. We also found that different adult attachment styles were associated with different kinds of emotional rewards. Findings support the theoretical utility of differentiating among several dispositional positive emotion constructs in personality research.

Scale:

The Big Five Inventory (BFI)

Here are a number of characteristics that may or may not apply to you. For example, do you agree that you are someone who likes to spend time with others? Please write a number next to each statement to indicate the extent to which you agree or disagree with that statement.

Disagree strongly	Disagree a little	Neither agree nor disagree	Agree a little	Agree Strongly
1	2	3	4	5

I see Myself as Someone Who...

____1. Is talkative

____2. Tends to find fault with others

____3. Does a thorough job

____4. Is depressed, blue

____5. Is original, comes up with new ideas

____6. Is reserved

____7. Is helpful and unselfish with others

____8. Can be somewhat careless

____9. Is relaxed, handles stress well

____10. Is curious about many different things

____11. Is full of energy

____12. Starts quarrels with others

____13. Is a reliable worker

____14. Can be tense

____15. Is ingenious, a deep thinker

____16. Generates a lot of enthusiasm

____17. Has a forgiving nature

____18. Tends to be disorganized

____19. Worries a lot

____23. Tends to be lazy

____24. Is emotionally stable, not easily upset

____25. Is inventive

____26. Has an assertive personality

____27. Can be cold and aloof

____28. Perseveres until the task is finished

____29. Can be moody

____30. Values artistic, aesthetic experiences

____31. Is sometimes shy, inhibited

____32. Is considerate and kind to almost everyone

____33. Does things efficiently

____34. Remains calm in tense situations

____35. Prefers work that is routine

____36. Is outgoing, sociable

____37. Is sometimes rude to others

____38. Makes plans and follows through with them

____39. Gets nervous easily

____40. Likes to reflect, play with ideas

____41. Has few artistic interests

____20. Has an active imagination

____21. Tends to be quiet

____22. Is generally trusting

____42. Likes to cooperate with others

____43. Is easily distracted

____44. Is sophisticated in art, music, or literature

Scoring:

BFI scale scoring ("R" denotes reverse-scored items):

Extraversion: 1, 6R, 11, 16, 21R, 26, 31R, 36
Agreeableness: 2R, 7, 12R, 17, 22, 27R, 32, 37R, 42
Conscientiousness: 3, 8R, 13, 18R, 23R, 28, 33, 38, 43R
Neuroticism: 4, 9R, 14, 19, 24R, 29, 34R, 39
Openness: 5, 10, 15, 20, 25, 30, 35R, 40, 41R, 44

2. The Weinberger Adjustment Inventory (WAI)

(Not shown here)

3. Toronto Alexithymia Scale (TAS- 20)

Toronto Alexithymia Scale (TAS-20)

The TAS-20 utilizes a five-point Likert scale with five of the items inversely scored. It is hand scored with a maximum score of 100. It uses cutoff scoring: equal to or less than 51 = non-alexithymia, equal to or greater than 61 = alexithymia. Scores of 52 to 60 = possible alexithymia. The maximum scores for each of the subscales are: Factor 1 (7 items): 35; Factor 2 (5 items): 25; Factor 3 (8 items): 40. There are no cutoff scores established for each of the three factor subscales.

F1 - Difficulty Identifying Feelings

1. I am often confused about what emotion I am feeling.
3. I have physical sensations that even doctors don't understand.
6. When I am upset, I don't know if I am sad, frightened, or angry.
7. I am often puzzled by sensations in my body.
9. I have feelings that I can't quite identify.
13. I don't know what's going on inside me.
14. I often don't know why I am angry.

F-2 - Difficulty Describing Feelings

2. It is difficult for me to find the right words for my feelings.
4. I am able to describe my feelings easily.
11. I find it hard to describe how I feel about people
12. People tell me to describe my feelings more.
17. It is difficult for me to reveal my innermost feelings, even to close friends.

F-3 - Externally-Oriented Thinking

5. I prefer to analyse problems rather than just describe them.
8. I prefer to just let things happen rather than to understand why they turned out that way.
10. Being in touch with emotions is essential.
15. I prefer talking to people about their daily activities rather than their feelings.
16. I prefer to watch "light" entertainment shows rather than psychological dramas.
18. I can feel close to someone, even in moments of silence.
19. I find examination of my feelings useful in solving personal problems.
20. Looking for hidden meanings in movies or plays distracts from their enjoyment.

Note: Items 4, 5, 10, 18, and 19 are inversely keyed.

Development of the TAS-20

In the absence of valid and reliable instruments to measure the alexithymia construct, Taylor, Ryan and Bagby (1985) devised the original self-report Toronto Alexithymia Scale (TAS). They used both empirical and rational methods in scale construction, initially defining five domains of alexithymia: (a) difficulty describing feelings, (b) difficulty distinguishing between feelings and accompanying bodily sensations, (c) lack of introspection, (d) social conformity, and (e) impoverished fantasy life and poor dream recall (Taylor et al., 1997). Responses were rated with a 5-point Likert scale ranging from 'strongly disagree' to 'strongly agree'. After factor and item analysis, the 41 item questionnaire was pared to 26 items and four domains that were more theoretically consistent with the alexithymia construct. These four domains, or factors, were: Factor 1 (F1) difficulty identifying and distinguishing between feelings and bodily sensations, Factor 2 (F2) difficulty describing feelings, Factor 3 (F3) reduced daydreaming, and Factor 4 (F4) externally oriented thinking.

While studies demonstrated support for the discriminant and convergent validity of the TAS, and the psychometric properties of the TAS were a considerable improvement over other available instruments, the construction of the TAS prompted refinement of the alexithymia construct and its essential facets. Daydreaming, for example, was determined to negatively correlate with the first factor (Taylor et al., 1997). In 1992 the attempt at scale reconstruction led to the development of a revised, 23 item self-report scale, the TAS-R. Continuing shortcomings with the scale prompted further examination of its compositional structure. There was high correlation between factors 1 and 2, and several items cross-loaded on each factor. Further revision resulted in the TAS-20 (Bagby, Parker &Taylor, 1994; Bagby, Taylor & Parker, 1994), a 20-item self-report scale consisting of 3 factors: Factor 1 (F1) difficulty identifying feelings, Factor 2 (F2) difficulty describing feelings, and Factor 3 (F3) externally-oriented thinking. These three factors represent essential intercorrelated traits that are theoretically congruent with the alexithymia construct. The TAS-20 eliminates the theoretical overlap of the three factors and the cross-loading of items that was a liability in earlier versions, and demonstrates good internal consistency and test-retest reliability. "The psychometric properties of the TAS provide considerable empirical support for the validity of the alexithymia construct" (Taylor et al., p. 49).

4. Hospital Anxiety and Depression Scale (HADS)

HAD SCALE

Name: ..... Date:.....

Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he will be able to help you more.

This questionnaire is designed to help your doctor to know how you feel. Read each item and place a firm tick in the box opposite the reply which comes closest to how you have been feeling in the past week.

Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought-out response.

Tick only one box in each section.

I feel tense or 'wound up: Most of the time ..... A lot of the time ..... Time to time, occasionally. Not at all		**I feel as if I am slowed down:** Nearly all the time ..... Very often ..... Sometimes ..... Not at all	
I still enjoy the things I used to enjoy: Definitely as much ..... Not quite so much ..... Only a little ..... Hardly at all		**I get a sort of frightened feeling like 'butterflies' in the stomach:** Not at all .... Occasionally ..... Quite often ..... Very often	
I get a sort of frightened feeling as if something awful is about to happen: Very definitely & quite badly Yes, but not too badly ..... A little, but it doesn't worry me. Not at all		**I have lost interest in my appearance:** Definitely .... I don't take so much care as I should I may not take quite as much care I take just as much care as ever	
I can laugh and see the funny side of things: As much as I always could Not quite so much now .... Definitely not so much now Not at all		**I feel restless as if I have to be on the move:** Very much indeed ..... Quite a lot ..... Not very much ..... Not at all	
Worrying thoughts go through my mind: A great deal of the time .... A lot of the time ..... From time to time but not too often ..... Only occasionally		**I look forward with enjoyment to things:** As much as ever I did ..... Rather less than I used to ..... Definitely less than I used to ... Hardly at all	
I feel cheerful: Not at all .... Not often ..... Sometimes ..... Most of the time		**I get sudden feelings of panic:** Very often indeed ..... Quite often ..... Not very often ..... Not at all	
I can sit at ease and feel relaxed: Definitely .... Usually..... ..... Not often ..... Not at all		**I can enjoy a good book or radio or TV programme:** Often .... Sometimes ..... Not often ..... Very seldom	

Do not write below this line

A - (8-10) .....

D - (8-10) .....

HAD SCALE SCORE SHEET

		A		D	
		3		3	
		2		2	
		1		1	
		0		0	
	D				A
	0				0
	1				1
	2				2
	3				3
		A		D	
		3		3	
		2		2	
		1		1	
		0		0	
	D				A
	0				3
	1				2
	2				1
	3				0
		A		D	
		3		0	
		2		1	
		1		2	
		0		3	
	D				A
	3				3
	2				2
	1				1
	0				0
		A		D	
		0		0	
		1		1	
		2		2	
		3		3	

FOR PHYSICIAN/ NURSE USE Patients Name/No:

D (8-10) .....

A (8-10) .....

HAD Scores of over 10, change of duties and refer to OHP
HAD Scores of over 21, ask whether panic attacks have occurred.

5. Spielberger State (SSAI) and Trait (STAI) anxiety Questionnaire

State-Trait Anxiety Inventory for Adults

Self-Evaluation Questionnaire

STAI Form Y-1 and Form Y-2

Developed by Charles D. Spielberger
in collaboration with R.L. Gorsuch, R. Lushene, P.R. Vagg, and G.A. Jacobs

Published by Mind Garden
1690 Woodside Road Suite 202, Redwood City, CA 94061 USA 650-261-3500
www.mindgarden.com

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:

Name_____Date_____S_____

Age_____ Gender (*Circle*) **M** **F** T_____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

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STAIP-AD Test Form Y
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SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name_____ Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

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STAIP-AD Test Form Y
www.mindgarden.com

State-Trait Anxiety Inventory for Adults Scoring Key (Form Y-1, Y-2)

Developed by Charles D. Spielberger in collaboration with R.L. Gorsuch, R. Lushene, P.R. Vagg, and G.A. Jacobs

To use this stencil, fold this sheet in half and line up with the appropriate test side, either Form Y-1 or Form Y-2. Simply total the scoring **weights** shown on the stencil for each response category. For example, for question # 1, if the respondent marked 3, then the **weight** would be **2**. Refer to the manual for appropriate normative data.

Form Y-1	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1.	4	3	2	1
2.	4	3	2	1
3.	1	2	3	4
4.	1	2	3	4
5.	4	3	2	1
6.	1	2	3	4
7.	1	2	3	4
8.	4	3	2	1
9.	1	2	3	4
10.	4	3	2	1
11.	4	3	2	1
12.	1	2	3	4
13.	1	2	3	4
14.	1	2	3	4
15.	4	3	2	1
16.	4	3	2	1
17.	1	2	3	4
18.	1	2	3	4
19.	4	3	2	1
20.	4	3	2	1

Form Y-2	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21.	4	3	2	1
22.	1	2	3	4
23.	4	3	2	1
24.	1	2	3	4
25.	1	2	3	4
26.	4	3	2	1
27.	4	3	2	1
28.	1	2	3	4
29.	1	2	3	4
30.	4	3	2	1
31.	1	2	3	4
32.	1	2	3	4
33.	4	3	2	1
34.	4	3	2	1
35.	1	2	3	4
36.	4	3	2	1
37.	1	2	3	4
38.	1	2	3	4
39.	4	3	2	1
40.	1	2	3	4

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STAIP-AD Scoring Key
www.mindgarden.com

6. Vulnerable Attachment Style Questionnaire (VASQ)

Vulnerable Attachment Style Questionnaire

FEELINGS ABOUT RELATIONSHIPS (VASQ)

Below are a number of statements concerning the way people feel about themselves in relation to others. Indicate whether you agree of disagree with the description as it applies to you by circling the answer that applies to you.

	Strongly Agree	Agree	Unsure	Disagree	Strongly disagree
1. I take my time getting to know people.	5	4	3	2	1
2. I rely on others to help me make decisions in life	5	4	3	2	1
3. People let me down a lot	5	4	3	2	1
4. I miss the company of others when I'm alone	5	4	3	2	1
5. Its best not to get too emotionally close to other people	5	4	3	2	1
6. I worry a lot if people I live with arrive back later than expected	5	4	3	2	1
7. I usually rely on advice from others when I've got a problem	5	4	3	2	1
8. I feel uncomfortable when people get too close to me	5	4	3	2	1
9. People close to me often get on my nerves	5	4	3	2	1
10. I feel people are against me	5	4	3	2	1
11. I worry about thinks happening to close family and friends	5	4	3	2	1
12. I often get into arguments	5	4	3	2	1
13. I'm clingy with others	5	4	3	2	1
14. I look forward to spending time on my own	5	4	3	2	1
15. I like making decisions on my own	5	4	3	2	1
16. I get anxious when people close to me are away	5	4	3	2	1
17. I feel uneasy when others confide in me	5	4	3	2	1
18. I find it hard to trust others	5	4	3	2	1
19 Having people around me can be a nuisance	5	4	3	2	1
20. I feel people haven't done enough for me	5	4	3	2	1
21. Its important to have people around me a lot of the time	5	4	3	2	1
22. I find it difficult to confide in people	5	4	3	2	1

Appendix Three

Explanatory note BFI:

A summary of the factors of the Big Five and their constituent traits:[1]

1. **Extraversion** – (outgoing/energetic vs. solitary/reserved). Energy, positive emotions, surgency, assertiveness, sociability and the tendency to seek stimulation in the company of others, and talkativeness.
2. **Agreeableness** – (friendly/compassionate vs. cold/unkind). A tendency to be compassionate and cooperative rather than suspicious and antagonistic towards others. It is also a measure of ones' trusting and helpful nature, and whether a person is generally well tempered or not.
3. **Conscientiousness** – (efficient/organised vs. easy-going/careless). A tendency to show self-discipline, act dutifully, and aim for achievement; planned rather than spontaneous behaviour; organized, and dependable.
4. **Neuroticism** – (sensitive/nervous vs. secure/confident). The tendency to experience unpleasant emotions easily, such as anger, anxiety, depression, or vulnerability. Neuroticism also refers to the degree of emotional stability and impulse control, and is sometimes referred by its low pole – "emotional stability".
5. **Openness to experience** – (inventive/curious vs. consistent/cautious). Appreciation for art, emotion, adventure, unusual ideas, curiosity, and variety of experience. Openness reflects the degree of intellectual curiosity, creativity and a preference for novelty and variety a person has. It is also described as the extent to which a person is imaginative or independent, and depicts a personal preference for a variety of activities over a strict routine. Some disagreement remains about how to interpret the openness factor, which is sometimes called "intellect" rather than openness to experience.

[1] Atkinson RL, Atkinson RC, Smith EE, Bem DJ, Nolen-Hoeksema S. Hilgard's Introduction to Psychology (13 ed.). Harcourt College Publishers. 2000: 437.

Appendix Four

1. Study 1 – Pilot Study: PNS modulation data on pain reporting

As part of examining the psychological dimension of the cohort in study 1, the volunteers' subjective reporting responses as measured by Visual Analogue Scale (VAS) scores during different phases of the experiment for pain and unpleasantness was recorded at baseline (T0), during the acid infusion, and for time points T60, T90 and T120, and compared between visits. With regards to the subjective pain experienced, there was no statistical difference between visits, but for the unpleasantness, there was some differences detected. Of note is the observation that immediately (T60) post Psychological stress induction (green graph, Figure ax-4.1(B)), the unpleasantness VAS score was the highest, 7.38 ±1.60(SD), p=0.053, but at T120, it was the lowest, 3.43 ±2.30(SD), p=0.019. [p values were calculated relative to there difference with regards to Screening visit] A second observation was that for both pain and unpleasantness during the acid infusion period, the Deep breathing protocol produced the lowest subjective ratings.

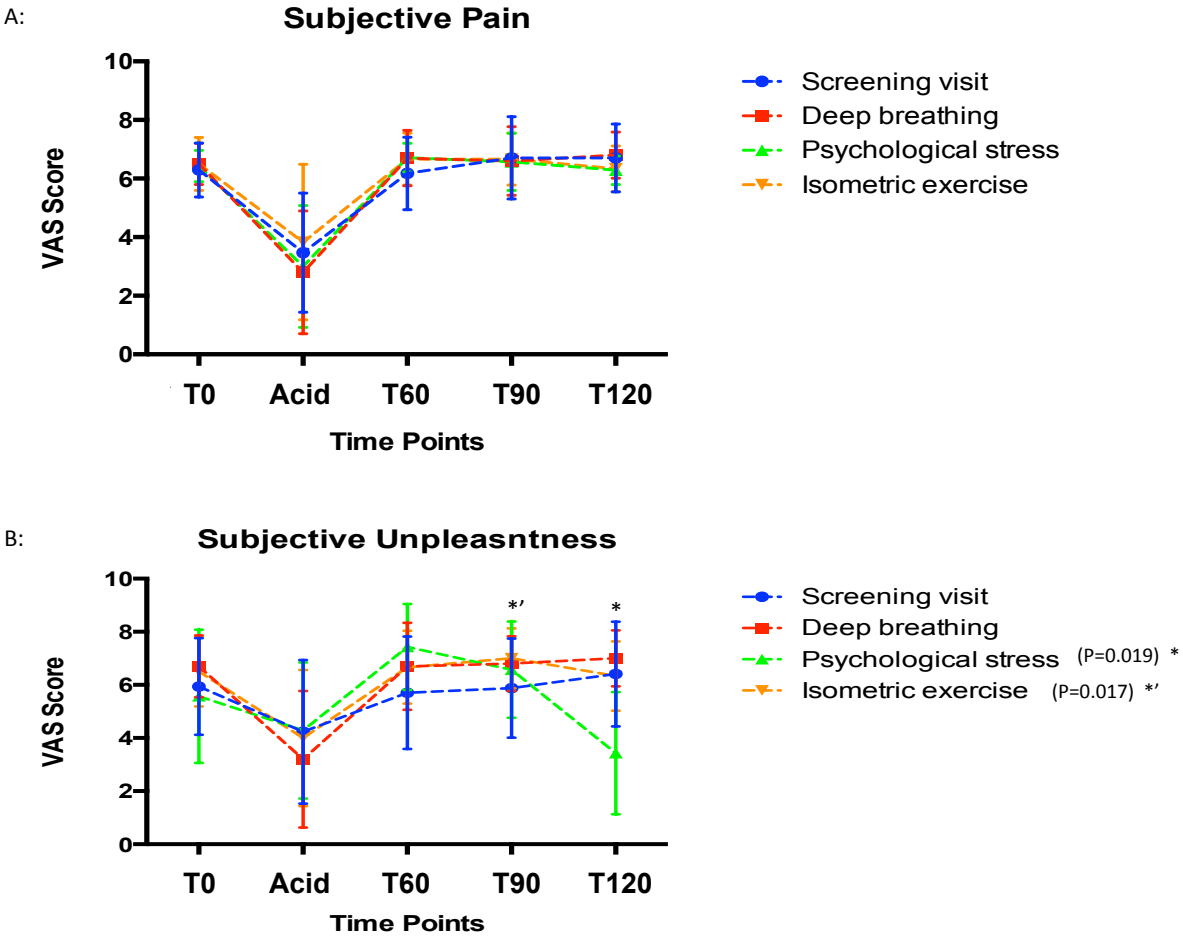
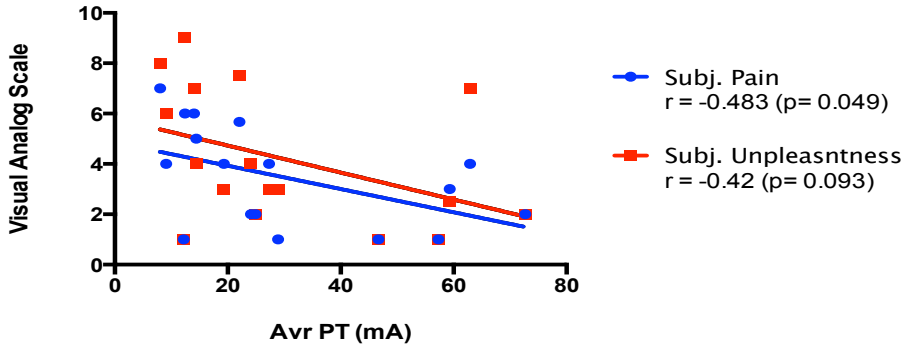


Figure ax-4.1: *Subjective rating **A:** experienced pain in the proximal oesophagus, and **B:** unpleasantness experienced due to the electrical sensitivity stimulus, and acid infusion.*

Looking at the relationship between subjective pain and unpleasantness reported, and the objective pain-stimulation strength actually experienced (experimentally delivered), correlations of note were detected. During Screening visit there was a negative correlation between the average pain threshold (Avr PT) and for both the reporting of pain, r=-0.483 (p=0.049) and unpleasantness, r=-0.42 (p=0.093). (Figure ax-4.2(A)) A similar finding was seen during the psychological stress induction, with regards to the difference in pain threshold (Δ PT) and the subjective reporting of pain, r=-0.787 (p=0.036) with unpleasantness, r=-0.849 (p=0.016). (Figure ax-4.2(B)) Further analysis revealed that the correlation was sensitive to the inclusion/exclusion of an outlying point.

When the analysis was repeated after the exclusion of the outlier there remained statistical significance for unpleasantness, r=-0.892 (p=0.017), but not for pain, r=-0.602 (p=0.206). These correlations imply that the lower the volunteers' pain threshold (Avr PT), or the higher there degree of sensitivity (ΔPT) were; the more likely they were of reporting the stimulus experienced as more painful or unpleasant.

A: **Correlation of average pain threshold (PT) and the Subjective Experiance during Screening visit protocol (n=17).**



B: **Correlation of diffirance in pain threshold (Δ PT) and the Subjective Experiance during Psychological stress protocol (n=7).**

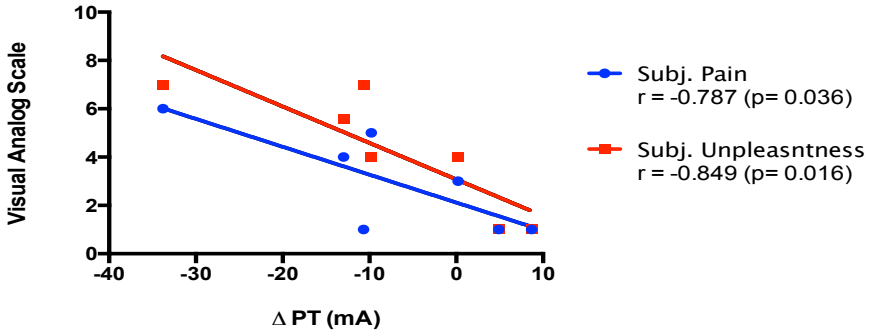


Figure ax-4.2: A: *The correlation between average pain threshold (Avr PT) and subjective reporting on a visual analogue scale (VAS) during screening visit.* **B:** *The correlation between the difference in pain threshold (ΔPT) and) and subjective reporting on a visual analogue scale (VAS) during psychological stress protocol.*

Study 1 – Pilot Study: Pain reporting data tables

A:

Subj. Pain		T0	Acid	T60	T90	T120
SV	AVR	6.28	3.45	6.13	6.72	6.70
	SD	0.87	2.01	1.20	1.41	1.16
	SEM	0.21	0.49	0.29	0.34	0.28
	n	17	17	17	17	17
DB	AVR	6.5	2.8	6.65	6.6	6.8
	SD	0.71	2.10	0.88	1.17	0.79
	SEM	0.22	0.66	0.28	0.37	0.25
	n	10	10	10	10	10
	p	0.496	0.916	0.244	0.999	0.619
ST	AVR	6.38	3.00	6.67	6.55	6.33
	SD	0.49	2.08	0.47	0.97	0.47
	SEM	0.18	0.79	0.18	0.36	0.18
	n	7	7	7	7	7
	p	0.231	0.067	0.103	0.111	0.134
HG	AVR	6.50	3.83	6.63	6.67	6.33
	SD	0.90	2.66	0.83	0.89	0.78
	SEM	0.26	0.77	0.24	0.26	0.22
	n	12	12	12	12	12
	p	0.423	0.245	0.253	0.740	0.491

B:

Subj. Un-pleasantness		T0	Acid	T60	T90	T120
SV	AVR	5.93	4.18	5.70	5.82	6.39
	SD	1.81	2.68	2.11	1.80	1.94
	SEM	0.44	0.65	0.51	0.44	0.47
	n	17	17	17	17	17
DB	AVR	6.65	3.20	6.60	6.80	6.95
	SD	1.11	2.57	1.60	1.03	1.01
	SEM	0.35	0.81	0.50	0.33	0.32
	n	10	10	10	10	10
	p	0.134	0.819	0.405	0.031 *	0.217
ST	AVR	5.50	4.23	7.38	6.62	3.43
	SD	2.47	2.52	1.60	1.83	2.30
	SEM	0.93	0.95	0.61	0.69	0.87
	n	7	7	7	7	7
	p	0.656	0.063	0.053	0.377	0.019 *
HG	AVR	6.46	4.00	6.63	7.00	6.29
	SD	1.27	2.56	1.33	1.13	1.25
	SEM	0.37	0.74	0.38	0.33	0.36
	n	12	12	12	12	12
	p	0.099	0.281	0.321	0.017 *	0.687

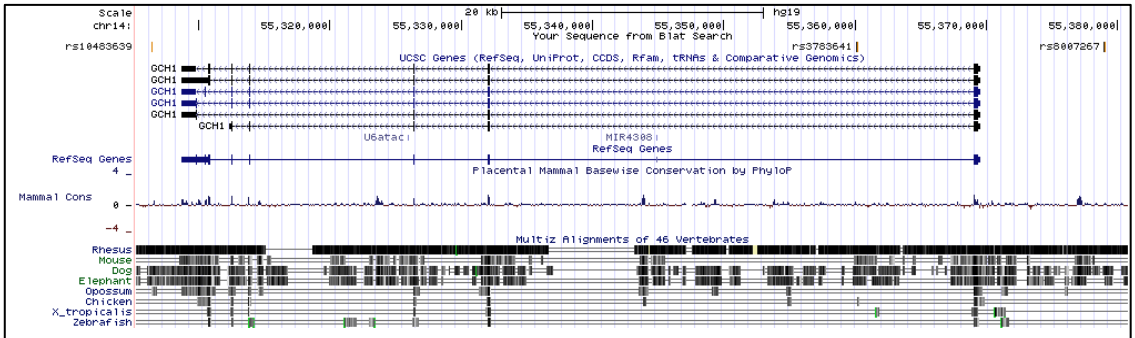
SV: Screening visit protocol

DB: Deep breathing protocol

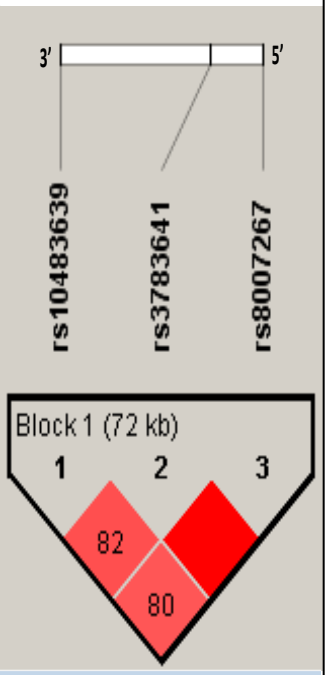
ST: Stress induction protocol

HG: Handgrip protocol

2. Study 5 – GCH-1 Genetic probe 'well – table':



	Genotypes			Haplotypes								ATG
				Most Probable				2nd Most Probable				
	55306432	55360114	55378966	H1	H2	p	H1	H2	p			
	rs10483639	rs3783641	rs8007267									
AM158	1 1	1 1	0 0	1 1	1 1	1				0/0		
AM101	1 2	1 1	1 1	1 1 1	2 1 1	1				0/0		
AM104	1 2	1 2	1 2	1 1 1	2 2 2	0.993	2 1 1	1 2 2	0.0068		O/X	
AM106	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM107	1 2	1 1	1 1	1 1 1	2 1 1	1				0/0		
AM109	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
						0.99314						
AM110	1 2	1 2	1 2	1 1 1	2 2 2	4	2 1 1	1 2 2	0.0068		O/X	
AM112	1 1	1 2	1 2	1 1 1	1 2 2	1				0/0		
AM113	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM115	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM116	1 2	1 2	1 2	1 1 1	2 2 2	0.993144	2 1 1	2 2 1	0.006856		O/X	
AM117	1 2	1 2	1 2	1 1 1	2 2 2	0.993144	2 1 1	2 2 1	0.006856		O/X	
AM118	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM119	1 2	1 1	2 2	1 1 2	2 1 2	1				0/0		
AM121	2 2	2 2	2 2	2 2 2	2 2 2	1						X/X
AM123	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM124	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM125	1 2	1 2	1 2	1 1 1	2 2 2	0.993144	2 1 1	2 2 1	0.006856		O/X	
						0.99314						
AM126	1 2	1 2	1 2	1 1 1	2 2 2	4	2 1 1	2 2 1	0.006856		O/X	
AM127	1 2	2 2	2 2	1 2 2	2 2 2	1					O/X	
AM128	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM129	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM130	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM133	0 0	1 2	1 2	0 1 1	0 2 2	1					O/X	
AM134	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM138	0 0	0 0	1 1	0 0 1	0 0 1	1				0/0		
AM141	1 2	1 2	1 2	1 1 1	2 2 2	0.993144	2 1 1	2 2 1	0.006856		O/X	
AM144	1 1	1 1	1 2	1 1 1	1 1 2	1				0/0		
AM146	1 1	1 1	1 2	1 1 1	1 1 2	1				0/0		
AM147	0 0	0 0	0 0									
AM148	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM150	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
AM151	1 1	1 1	1 2	1 1 1	2 1 1	1				0/0		
AM152	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
						0.99314						
AM153	1 2	1 2	1 2	1 1 1	2 2 2	4	2 1 1	2 2 1	0.006856		O/X	
						0.99314						
AM155	1 2	1 2	1 2	1 1 1	2 2 2	4	2 1 1	2 2 1	0.006856		O/X	
AM162	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		
						0.99314						
AM164	1 2	1 2	1 2	1 1 1	2 2 2	4	2 1 1	2 2 1	0.006856		O/X	
AM166	1 1	1 1	1 1	1 1 1	1 1 1	1				0/0		

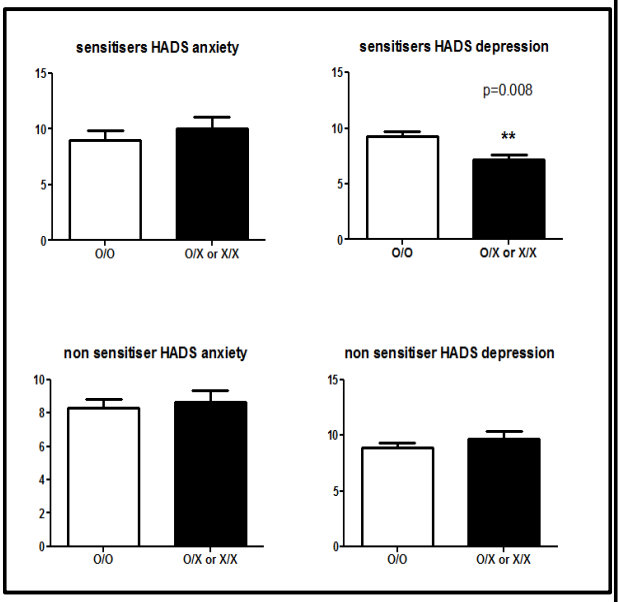


Haplotype Frequecies

Haplotype	Haploview	
	Obs	Freq
1111		0.69
2222		0.19
3112		0.06
4122		0.03
5211		0.03
6212		0.01
7110		
8011		
9022		
10001		

3. Study 5 - HADS profiling data:

n	status	number	HADS Anxiety	Depression
1	Non sensitisers	1	9	8
2	Non sensitisers	7	6	8
3	Non sensitisers	13	12	9
4	Non sensitisers	24	6	11
5	Non sensitisers	25	9	8
6	Non sensitisers	26	9	9
7	Non sensitisers	29	7	9
8	Non sensitisers	30	10	8
9	Non sensitisers	33	8	6
10	Non sensitisers	34	11	13
11	Non sensitisers	41	8	13
12	Non sensitisers	48	7	9
13	Non sensitisers	50	6	8
14	Non sensitisers	52	9	10
15	Non sensitisers	55	6	8
16	Non sensitisers	58	10	9
17	Non sensitisers	62	10	10
18	Non sensitisers	64	8	9
19	Non sensitisers	66	9	8
		avr	8.42	9.11
		SD	23.45	23.45
		SEM	5.38	5.38
1	Sensitisers	4	13	7
2	Sensitisers	6	7	8
3	Sensitisers	9	5	9
4	Sensitisers	10	9	9
5	Sensitisers	12	10	10
6	Sensitisers	15	8	9
7	Sensitisers	16	7	6
8	Sensitisers	17	11	7
9	Sensitisers	18	9	9
10	Sensitisers	19	12	13
11	Sensitisers	21	10	6
12	Sensitisers	23	12	11
13	Sensitisers	27	14	6
14	Sensitisers	28	14	7
15	Sensitisers	38	8	9
16	Sensitisers	44	4	8
17	Sensitisers	46	9	9
20	Sensitisers	53	6	9
		avr	9.33	8.44
		SD	2.95	1.82
		SEM	0.68	0.42
		t test: p=	0.259	0.265



n	status	number	HADS Anxiety	Depression
(O/O)				
1	Sensitisers	4	13	7
4	Sensitisers	10	9	9
7	Sensitisers	16	7	6
8	Sensitisers	17	11	7
11	Sensitisers	21	10	6
13	Sensitisers	27	14	6
20	Sensitisers	53	6	9
			10	7.14
(O/X,X/X)				
2	Sensitisers	6	7	8
3	Sensitisers	9	5	9
5	Sensitisers	12	10	10
6	Sensitisers	15	8	9
9	Sensitisers	18	9	9
10	Sensitisers	19	12	13
12	Sensitisers	23	12	11
14	Sensitisers	28	14	7
15	Sensitisers	38	8	9
16	Sensitisers	44	4	8
17	Sensitisers	46	9	9
19	Sensitisers	51	10	9
			9	9.25
		t test: p=	0.480	0.008

Soli Deo Gloria